Neuromuscular features of Ehlers-Danlos syndrome and Marfan syndrome

expanding the phenotype of inherited connective tissue disorders and investigating the role of the extracellular matrix in muscle

Nicol Voermans

The research in this thesis was performed a the Radboud University Nijmegen Medical Centre in Nijmegen, at the Faculty of Movement Sciences of the Free University in Amsterdam, and at the Leiden University Medical Centre in Leiden. N.C. Voermans was financially supported by the Dutch Organisation for Scientific Research (NWO AGIKO grant no.920-03-378).

The publication of this thesis was financially supported by the Department of Neurology of the Radboud University Nijmegen Medical Centre, the Donders Centre for Brain, Cognition and Behaviour, the Institute for Fundamental and Clinical Human Movement Sciences (IFKB), UCB Pharma BV, and Genzyme.

Cover design and layout by In Zicht Grafisch Ontwerp, www.promotie-inzicht.nl
Printed by Ipskamp Drukkers B.V., www.ipskampdrukkers.nl

ISBN 978-90-9026238-3

Neuromuscular features of Ehlers-Danlos syndrome and Marfan syndrome

expanding the phenotype of inherited connective tissue disorders and investigating the role of the extracellular matrix in muscle

Een wetenschappelijke proeve op het gebied
van de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op donderdag 22 september 2011
om 10.30 uur precies

door

Nicol Cornelia Voermans
geboren op 14 juni 1973
te Zetten

Promotoren

Prof. dr. B.G.M. van Engelen

Prof. dr. B.C. Hamel

Prof. dr. A. de Haan (VU)

Manuscriptcommissie

Prof. dr. M.T.E. Hopman, voorzitter

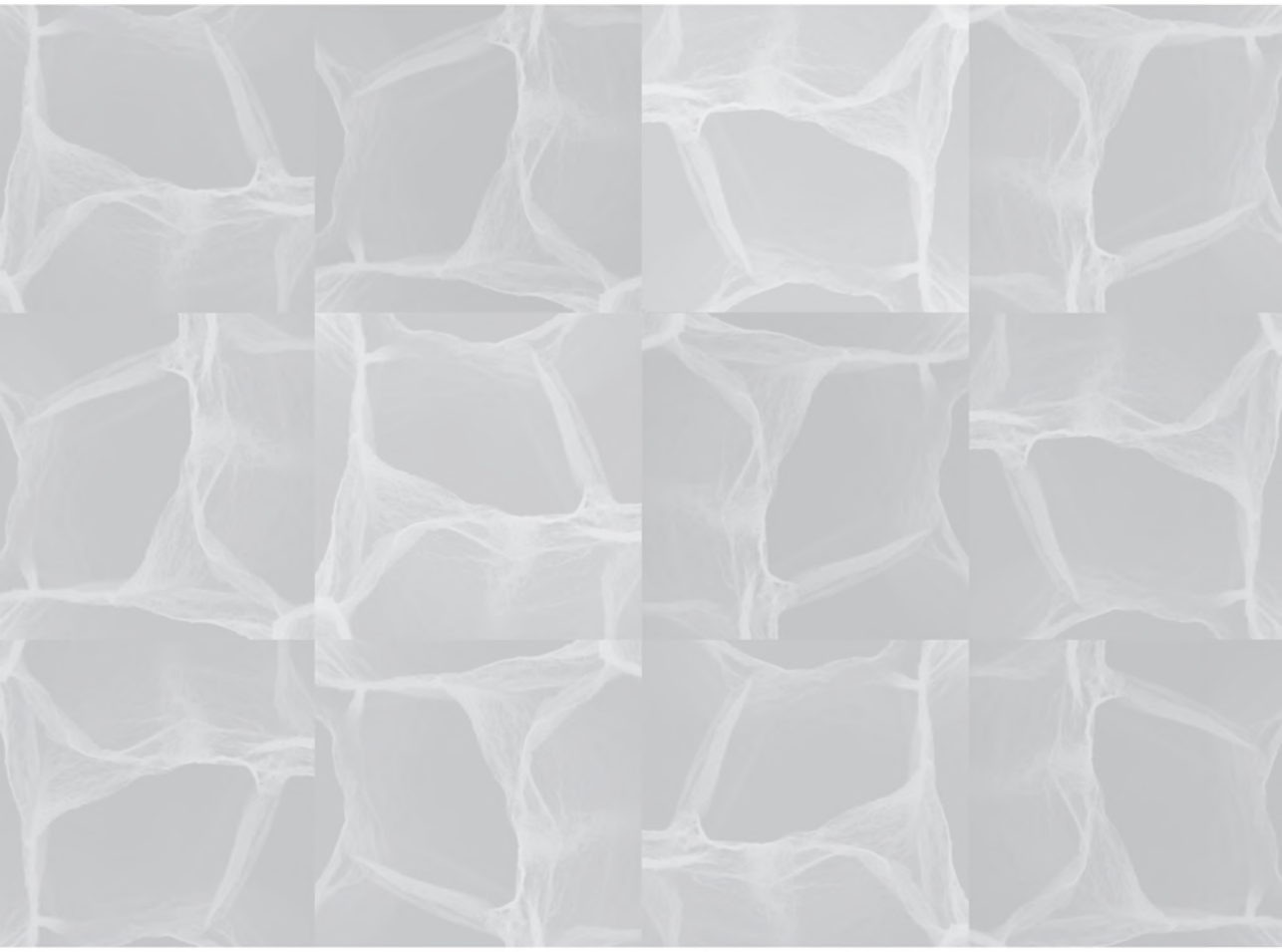
Prof. dr. H.P. Kremer (UMC Groningen)

Prof. dr. B.L. Loeys (Universiteit Gent)

Contents

PART

Introduction and outline



CHAPTER 1

General introduction and outline of this thesis

The first chapter of this thesis presents an introduction with a description of the background, main concepts, aims, and outline of this study.

General introduction

Ehlers-Danlos syndrome (EDS) and Marfan syndrome are the two most common inherited connective tissue disorders (ICTDs).[1,2] They are characterized by a wide variety of dermal, articular, orthopaedic, gastrointestinal, gynaecological, vascular, and cardiac symptoms due to an abnormal composition of the connective tissue. Neuromuscular symptoms, such as muscle weakness, fatigue, exercise intolerance, and muscle cramps are only sporadically reported and generally understood as secondary to reduced physical activity.

Our clinical interest in the neuromuscular features in EDS and Marfan syndrome was raised by the encounter with these patients at our outpatients department. They were referred diagnostic work-up with complaints of muscle weakness, fatigue or exercise intolerance, and pain (*Box 1*). Ancillary investigations revealed no signs of a concomitant neuromuscular disorder, although these patients displayed mild muscle weakness or sensory disturbances or both. These encounters have put forth the hypothesis that neuromuscular symptoms might be an intrinsic part of the phenotype of these multisystem disorders. With a dense network of connective tissue present within muscle and peripheral nerve, this seems a plausible assumption.

Recent developments in neuromuscular research have further increased our attention on neuromuscular features in EDS and Marfan syndrome. Myopathies are generally understood to be caused by defects or deficiencies of molecules inside muscle cells or along the muscle membrane (sarcolemma). Over the last few years however, a number of myopathies have been found to be caused by mutations in the gene encoding the extracellular matrix (ECM) molecule collagen VI; both Bethlem myopathy and Ullrich Congenital Muscular Dystrophy (UCMD) are caused by mutations in collagen VI.[3] Apparently, a primary change in the ECM which surrounds muscle cells is capable of influencing muscle function drastically. At the same time, these developments have raised our scientific interest into the physiological effect of alterations of the muscle ECM on quantitative muscle function. This interest has grown with current investigations on myofascial force transmission,[4] which suggests that the connective tissue within and between the muscles plays a role in force transmission.

Following the above, the focus of this thesis is dual. First, it aims to explore the occurrence of neuromuscular symptoms in two ICTDs: EDS and Marfan syndrome. Second, it endeavours to elucidate the role of the ECM in muscle function by investigating how quantitative muscle function changes in EDS. We will explain this inspiration and focus in this introduction. Next, we will present the objectives of this study and give an outline of the thesis.

BOX 1 Three patients with Ehlers-Danlos syndrome.

Patient 1
A 58-year old male patient was referred for analysis of proximal weakness. EDS was diagnosed at the age of 46 following a knee haemorrhage. He also suffered from frequent subluxations of his shoulders, easy bruising, and hyperextensible skin. Later this was specified as the tenascin X (TNX)-deficient type EDS. His medical history further showed a gastric haemorrhage due to ulcers, mitral valve endocarditis and prolaps, renal insufficiency, and bilateral pneumothorax. Motor milestones were not delayed, but he had never been very good at sports. He reported limited physical fitness, reduced strength, and easy fatigability compared with peers. Walking stairs was difficult due to weakness and dyspnoea. Physical examination revealed generalized joint hypermobility and a hyperextensible skin. Proximal muscle strength in arms was MRC 4/5, in legs MRC 3/5, and distal weakness was very mild (MRC 4/5). Creatine kinase was normal. Electromyography revealed myopathic units in proximal arm and leg muscles. Muscle biopsy revealed myopathic changes without signs of a specific myopathy. At the age of 58, he was admitted at the ICU after replacement of the mitral valve. This was complicated by perforation of the piriformis sinus during introduction of a gastric tube. Subsequently, he developed sepsis with multi-organ failure and died.
Patient 2
A 42-year old female patient was referred for analysis of proximal muscle weakness and impairments in daily life. Hypermobility type EDS was diagnosed at the age of 41. Motor development was normal, and she had been active in sports until adolescence. Her complaints started eight years ago, when she remarked soreness of her leg muscles after strenuous physical activity. Since an uterus extirpation seven years ago, she suffered from recurrent subluxations of her right hip, and walking became impaired. Walking distance was now limited to 10 minutes due to pain in her feet, in spite of orthopedic shoes. Riding a bicycle was only possible for 1 km due to difficulties holding the steer and fatigue in her legs. Driving a car with cruise control was possible for short distances only. She worked part time (50%) as a secretary at a social service and used voice recognition software. An extensive rehabilitation program focusing on improvement of her physical condition and increase of muscle strength had increased her articular complaints, but had not resulted in improvement of muscle strength. Physical examination showed generalized joint hypermobility, mild proximal hip girdle weakness, and weakness in hands, wrists, and foot extensors (MRC 4). Provocation test showed no signs of myasthenia. Creatine kinase was normal. Muscle ultrasound revealed mild increase of ultrasound intensity of the gastrocnemius muscle and carpal flexor muscles. Nerve conduction studies and electromyography were normal. She was again referred to a rehabilitation centre, focusing on aids for her impairments and on finding a balance between her physical capacities and her daily activities, with good effect.
Patient 3
A 32-year old female patient was referred for analysis of her exercise intolerance and mild distal muscle weakness. EDS of the hypermobility type was diagnosed at the age of 25. Three years later, cervical dystonia was diagnosed in her and in her father, but no *DYT-11* mutation was detected. Motor development was normal. During childhood, she suffered from easy bruising and frequent (sub)luxations. Exercise tolerance had always been reduced in comparison with peers. An extensive training program in a rehabilitation centre had not improved that. Fatigue had been a prominent complaint since adolescence. She slept well and was well rested in the morning, but fatigued easily

BOX 1 Continued.

during the day. She worked part time as a secretary at a law firm (60%) but was exhausted after work and had no energy left to participate in any recreation or social activities. Her main concern was the complete lack of knowledge of EDS among the health insurance doctors she had encountered. Physical examination revealed mild cervical torticollis, generalized joint hypermobility, and mild weakness of finger extensors muscles and of dorsal interosseous muscles on both sides. Creatine kinase was normal. Nerve conduction studies showed no signs of ulnar neuropathy. Muscle ultrasound revealed increased ultrasound intensity of the tibial anterior muscle and biceps brachii muscle. Needle biopsy was not performed. Psychological screening consisting of standardized questionnaires pointed to severe fatigue and severe impairments in daily functioning.
She was reassured with the explanation of the results of recent studies on neuromuscular features, pain, fatigue, and natural course of the hypermobility type of EDS.

Discussion

Patient 1 is one of the first patients who was referred to our department for neuromuscular work-up and as such contributed to the initiative of this study. His case is descriptive since the TNX-deficient type EDS can be accompanied by mild to moderate proximal muscle weakness and polyneuropathy, while no signs of a concomitant myopathy or underlying cause for a polyneuropathy were found. Furthermore, we have recently reported another severe complication during ICU admittance in a patient with this type EDS.[5]
Patient 2 and 3 are illustrative for the subsequent referrals of EDS patients to our department. The hypermobility type EDS is the most common type EDS, and most patients are female. Mild proximal and/or distal muscle weakness usually manifests after adolescence. Fatigue is a prominent feature. Ancillary investigations generally show mild abnormalities, but no signs of overt myopathy. Complaints of exercise intolerance and fatigue are often neglected by medical specialists and in insurance procedures. Rehabilitation programs focusing at improving muscle strength often result in increase of articular problems rather than in functional improvement. Furthermore, it has been shown that the lack of knowledge of EDS among physicians and the resulting memory of not being respected is substantial for individuals with EDS and can last for years.[6,7]

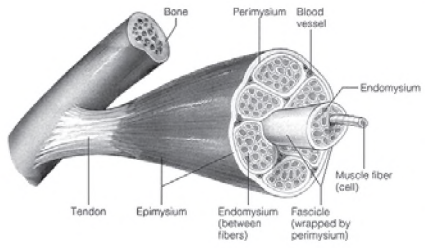
Connective tissue within muscle

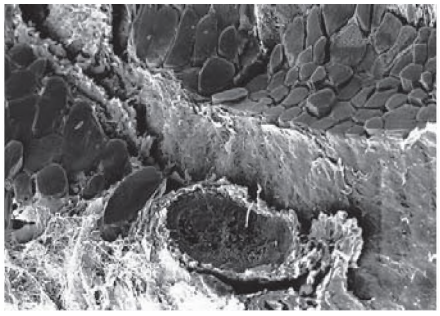
Connective tissue is any type of biological tissue that forms a network composed of the ECM and of nerve branches, capillaries, fibroblasts, and macrophages that are embedded within this matrix. It supports, binds together, and protects virtually all organs. The ECM network is built up of two major structural protein molecules: collagen and elastin. Collagen is the main component of connective tissue, and is the most abundant protein in mammals, making up about 25% to 35% of the whole-body protein content. It forms elongated fibrils which are mostly found in fibrous tissues such as tendon, ligament and skin. Collagen generally constitutes 1% to 2% of muscle tissue, and accounts for 6% of the weight of strong, tendinous muscles. Elastin is the elastic protein in connective tissue which allows many tissues in the body to resume their shape after stretching or contracting. For example, elastin helps skin to return to its original position when it is poked or pinched. Elastin is also an important load-bearing tissue and used in places where mechanical energy is required to be stored, such as in fascia and tendons.

Within muscle, connective tissue consists of endomysium, perimysium, and epimysium, surrounding the muscle fibres, fascicles, and the entire muscle respectively (*Figure 1, 2*). These connective tissue membranes are continuous with the tendons that connect the muscle to the bones. This continuity gives support to the muscle cells and enables a strong connection between the muscles and the bones, necessary to transmit force and to enable physiological movements. In general, the role of connective tissue within muscle has long been considered to be only mechanically supportive.

Figure 1 Cross section of a muscle with the connective tissue within muscle consisting of endo-, peri-, and epimysium. Reprinted with permission of the artist (K.Mount, www.gm-studio.com).

Figure 2 Close-up of a piece of pork muscle: muscle fibres (dark grey) surrounded by the perimysium (light grey). Reprinted with permission of the photographer: D. Marshall and D. Gregory, Wellcome Images, London, UK (http://www.wellcome.ac.uk/en/bia/gallery.html).





However, recent insights underscore the importance of the ECM in developmental and regenerative processes, as well as in transmembrane signalling in various tissues.[8,9] Hence, the ECM within muscle is gradually becoming recognized as a dynamic complex of molecules that interacts with sarcolemmal, cytoskeletal, and nuclear elements in order to maintain skeletal muscle integrity and to transmit forces.[10] In fact, the muscle ECM is a very dynamic structure that easily adapts to changes in physiological demand. Constant remodelling modifies the mechanical and viscoelastic properties, decreases stress-susceptibility, and may increase load-resistance.[11]

A shift of focus in neuromuscular research

The focus in neuromuscular research has gradually extended in line with these developments from the nucleus, contractile elements, and sarcolemma towards the ECM.[12] The novelty of

this development is reflected by the lack of detail in which the ECM has generally been depicted in reviews on myopathies and muscular dystrophies so far (*Figure 3A*). Our review in *Chapter 2*, focusing on the myopathies and the ICTDs caused by defects or deficiencies of ECM molecules, presents a more detailed image of the ECM within muscle (*Figure 3B*).

As a result of this broadening of the scope in neuromuscular research, a number of myopathies have been identified that result from deficiencies of ECM molecules or trans-sarcolemmal molecules projecting into the ECM: Bethlem myopathy and UCMD are caused by mutations in the gene encoding collagen VI. These collagen VI myopathies are characterized by hypotonia, delayed motor milestones, and a variable degree and pattern of muscle weakness. In addition, abnormal scar formation, soft velvety skin, and a combination of joint contractures and joint hypermobility is observed. Limb girdle muscular dystrophy 2E with joint hyperlaxity and contractures is associated with mutations in *SGCB* encoding the transsarcolemmal molecule beta-sarcoglycan.[3,13,14] The dermal and articular features in these myopathies point to a clinical overlap with ICTDs such as EDS and Marfan syndrome.[15,16] In fact, joint hypermobility can be useful in the differential diagnosis of myopathies.[17]

The role of the extracellular matrix in muscle function

The finding that a number of myopathies is caused by a deficiency of ECM molecules has raised our curiosity into the role of the ECM in muscle function. As a result, we became interested in studying the physiological mechanism in which alterations of the muscle ECM cause abnormal muscle function while the contractile elements within muscle cells seem to function normally. Recent research on myofascial force transmission offers a possible explanation; we will discuss this below.

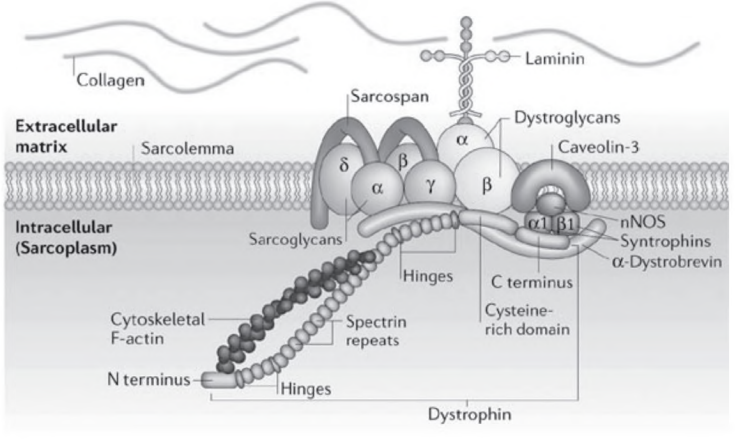
The general view of skeletal muscle is that force is generated within its muscle fibres and then directly transmitted in-series, usually via tendon, onto the skeleton. However, muscle fibres are mechanically connected to surrounding structures and cannot be considered as independent actuators. This suggests that the connective tissue within and between the muscles plays a role in force transmission. Indeed, force transmission occurs not only through myotendinous pathways, but a proportion of the muscle force generated within a muscle is transmitted to the skeleton through intra-, inter- and extramuscular myofascial force transmission (*Box 2*). Within muscles this occurs via the endo- and perimysium with its intramuscular continuations of neurovascular tracts in which nerve branches and blood vessels are embedded. The epimysium and fascia between muscle enable extramuscular force transmission. Force transmission via these pathways might be influenced by alterations of the ECM.

Inherited connective tissue disorders

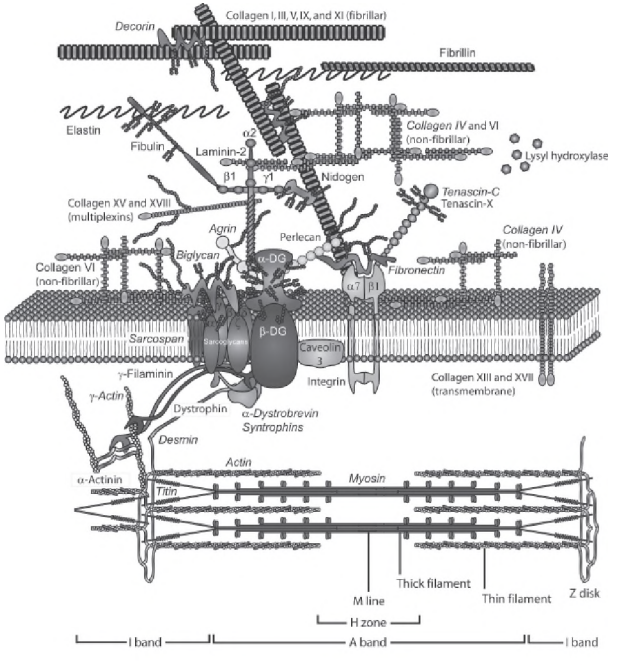
ICTDs are a group of inherited multisystem disorders due to abnormal composition of the connective tissue. These disorders are characterized by a varying degree of dermal, skeletal,

Figure 3 The focus in neuromuscular research has gradually extended from the nucleus, contractile elements, and sarcolemma towards the ECM. The novelty of this development is reflected by gradual increase of detail in which the ECM is being depicted in reviews.

A: Image in a review on muscular dystrophies in 2006, representative of most reviews so far: the ECM is depicted as coiled lines representing the collagen fibres in the ECM. The transsarcolemmal molecules are presented in detail with their subunits whereas the collagens are depicted only collectively as curled lines. Reprinted by permission from Macmillan Publishers Ltd: Nat Rev Mol Cell Biol, copyright 2006.[30]

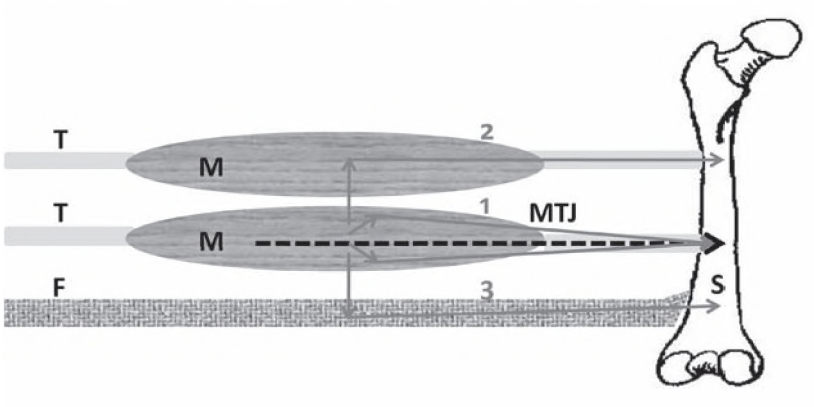


B: Image adapted from the image in our review on the clinical and molecular overlap of myopathies and inherited connective tissue disorders in 2008. The ECM is drawn in much more detail (DG = dystroglycan).[31]



BOX 2 Myofascial force transmission.

Muscle force is transmitted from the muscle fibre (M) in which it is generated onto the myotendinous junction (MTJ), and subsequently to the tendon (T) and skeleton (S): **myotendinous force transmission** (dashed line).
In addition, muscle force can also be transmitted through serial myofascial pathways:
1. Intramuscular myofascial force transmission: Force generated within muscle fibres can be transmitted via the network of intramuscular connective tissue.
Muscle force can also leave the muscle to be transmitted to the skeleton: Two epimuscular pathways are distinguished:
2. Intermuscular force transmission: force transmission between two neighbouring muscles via the continuous intermuscular connective tissue at their muscle belly interface, and
3. Extramuscular: force transmission between a muscle and adjacent non-muscular structures: extramuscular connective tissue of fascia, septa or neurovascular tracts (F).[4,21]



Myofascial force transmission thus contributes significantly to the total force generated in a muscle. Up to 40% of muscle force can be transmitted in this way.[22] Result of several studies on myofascial force transmission call for a new view of the locomotor apparatus, which needs to take into account the high interdependence of muscles and muscle fibres as force generators, as well as proximo-distal force differences and serial and parallel distributions of sarcomere lengths that are consequences of such interaction. If this is done, the effects of integrating a muscle fibre, muscle or muscle group into higher levels of organization of the body will become more clear.[23]

articular, orthopaedic, cardiovascular, gynaecological, and gastrointestinal symptoms. EDS and Marfan syndrome are the two most common ICTDs. They are caused by defects of collagen or TNX (EDS) and fibrillin (Marfan syndrome), the latter of which is one of the constituents of elastin. Among other ICTDs are osteogenesis imperfecta, cutis laxa, and Stickler syndrome.

Ehlers-Danlos syndrome

The key features of EDS are joint hypermobility, skin hyperextensibility, and tissue fragility, resulting in easy bruising, abnormal scarring, and arterial rupture (*Figure 4*). The overall prevalence of this condition has been estimated at 1:5-10,000.[18]

Figure 4 Clinical features of EDS.

A and **B**: Skin hyperextensibility and joint hypermobility in a 20-year-old male TNX-deficient EDS patient. **C**: Widened atrophic scars in a 34-year-old female patient with the classical type EDS.

Six major types of EDS are identified based upon clinical diagnostic criteria: the classical, hypermobility, vascular, kyphoscoliotic, arthrochalasia, and dermatosparaxis type, the latter three of which are very rare.[1] In 2001, a clinically and genetically distinct type was identified, the TNX-deficient type.[19] *Table 1* in *Chapter 4* presents these diagnostic criteria. The clinical variability of each EDS subtype is extremely wide and the diagnosis is not always straightforward even for the experienced clinician.[20] As a result, misdiagnosis or lack of diagnosis represents a major burden for patients with EDS.[7] In fact, a recent survey by the The European Organization for Rare Diseases (http://www.eurordis.org) has demonstrated that among patients belonging to 16 major rare diseases, those affected with EDS have the longest delay in diagnosis and request consultation of up to 20 specialists before obtaining the correct diagnosis.

This has severe consequences on the quality of life of the patients, usually in terms of excessive financial and time expense, superfluous investigations, wrong therapies, delay of appropriate treatments, and preventable worsening of the disease state.[20] Furthermore, it influences the attitude of patients towards medicine.[7] This is illustrated by a recent patients' account of their disease in the British Medical Journal.[6] These stories of three patients are similar in that symptoms have long been misunderstood, misinterpreted, and mishandled, and that their condition remained undiagnosed throughout childhood and adolescence.

The hypermobility type EDS is inherited as a dominant trait and is the most common type, with extreme hypermobility as its hallmark, and severe pain and fatigue developing later in life.[24] The classical type is also autosomal dominantly inherited and forms the second

most common type. Together with the hypermobility type, it accounts for approximately 90% of all EDS patients. It is characterized by joint hypermobility, skin hyperextensibility, and increased tissue fragility resulting in widened, atrophic scars. The vascular type EDS has thin, translucent skin, arterial/intestinal/uterine fragility or rupture, and extensive bruising as major diagnostic criteria. The phenotype of the TNX-deficient type is similar to that of the classical type, but inheritance is autosomal recessive and scars are not widened. The other types are much less common.

Muscle involvement in EDS can be expected based on interactions between muscle and the ECM molecules involved in the pathophysiology of EDS. Furthermore, muscle hypotonia and muscle rupture are part of the diagnostic criteria of EDS, and fatigue, musculoskeletal pain, and delayed gross motor development are described as associated features.[1] However, muscle symptoms such as muscle weakness and exercise intolerance are only sporadically reported in case reports , and often explained as a result of exercise avoidance due to joint hypermobility.[1,18,25,26]

Marfan syndrome

Marfan syndrome is characterized by ocular, skeletal, and cardiovascular manifestations, with an estimated prevalence of 1 in 3-5,000.[2] Mutations in the fibrillin-1 (*FBN1*) gene located at 15q21.1 account for most of the cases.[27] *FBN1* encodes fibrillin-1, a widely distributed major component of microfibrils in the ECM. It has an important role in elastin deposition in elastic fibres. Joint hypermobility is usually most pronounced in distal joints and often accompanied by arachnodactyly ('spider fingers') (*Figure 5*). (Congenital) joint contractures, particularly of the elbow, occur with moderate frequency.[2]

Figure 5 Clinical features of Marfan syndrome.

A and **B**: Arachnodactyly with positive thumb sign in a 27-year-old male Marfan syndrome patient. **C:** Distal joint hypermobility in a 28-year-old male Marfan syndrome patient.

Marfan patients frequently report muscle fatigue, and to a lesser extent muscle weakness, muscle hypoplasia, myalgia, and cramps, but these features have long been neglected in clinical practice and research.

Aims of this study

These observations in EDS and Marfan patients, together with the developments in neuromuscular and experimental research described above have led to the design of this study on neuromuscular features in EDS and Marfan syndrome and on the role of the ECM in muscle function.

Theoretically, we could have investigated the role of the ECM in muscle function in patients with collagen VI myopathies (Bethlem myopathy and UCMD). This might even seem a more plausible approach, since muscular features are very apparent in these myopathies caused by an ECM defect. However, as described above, EDS patients with muscular symptoms were referred to our outpatients department and formed the inspiration for this study. In parallel, recent experimental studies have shown that the pathophysiology of Bethlem myopathy and UCMD involves various intracellular changes: mitochondrial dysfunction and spontaneous apoptosis, leading to myofibre degeneration.[28] This probably results from defective autophagy.[29] These intracellular processes are likely to significantly influence muscle function. Therefore, collagen VI myopathies were not considered to be a very suitable model to investigate the role of the ECM on muscle function.

In order to explore the occurrence of neuromuscular symptoms in EDS and Marfan syndrome and to study the role of the ECM in muscle function this thesis has the following aims (numbering corresponds with the parts of the outline):

(I) to present an overview of the clinical and molecular overlap of the ICTDs and myopathies;
(IIA) to study the occurrence and nature of neuromuscular features in EDS;
(IIB) to investigate the pathophysiological mechanisms of muscle weakness in EDS in order to explore the role of the ECM in muscle function, and;
(III) to examine the occurrence of neuromuscular features in Marfan syndrome, to find out whether the findings in part IIA are specific for EDS or can also be found in other ICTDs.
Table 1 gives a schematic representations of the content of this thesis.

Outline of this thesis

Part I: Introduction and outline

The first part of this thesis presents an introduction with a description of the main concepts, aims of the study, and outline (*Chapter 1*); and deals with the overlap of ICTDs and myopathies (*Chapter 2*). *Chapter 2* presents an overview of neuromuscular involvement in ICTDs and of

myopathies with symptoms typically seen in ICTDs. As such, this review focuses on the molecular and clinical overlap between these disorders and myopathies. These ICTDs are caused by defects of matrix-embedded ECM molecules that are also present within muscle (collagen I, III, V, IX, lysylhydroxylase, tenascin, fibrillin, fibulin, elastin, and perlecan), and interact with trans-membrane glycoproteins. Insight in this clinical overlap can contribute to enhanced recognition of joint hypermobility as a distinctive feature in the differential diagnosis of myopathies.

Part II: Neuromuscular features of Ehlers-Danlos syndrome

Part IIA: Clinical evaluation of Ehlers-Danlos syndrome patients

In part IIA, the reports of our initial clinical observations and the results of the subsequent systematic clinical and questionnaire studies on neuromuscular involvement, fatigue, and pain in EDS are presented.

Chapter 3 consists of three case reports of EDS patients with neuromuscular involvement. First, we describe a patient with the hypermobility type EDS who subsequently presented with an axillary neuropathy, a brachial plexopathy, and a sciatic neuropathy. We discuss the possible pathophysiological mechanisms. The second report in this chapter describes the neuromuscular features of an adolescent patient with the kyphoscoliotic type of EDS, consisting of myopathy and polyneuropathy. Muscle hypotonia and weakness have so far only been recognized in neonates, but has not been reported later in life. In the third report, we present a TNX-deficient type EDS patient, who presented with moderate proximal and severe distal muscle weakness. The muscle atrophy and contractures in her hands point to the clinical overlap with Bethlem myopathy described in part I of this thesis.

Chapter 4 is an observational study on neuromuscular features in four types of EDS. Standardized questionnaires, physical examination, laboratory investigations, nerve conduction studies, electromyography, muscle ultrasound, and muscle biopsy were performed in 40 EDS patients with the vascular, classical, TNX-deficient type EDS, and hypermobility type of EDS due to *TNXB* haploinsufficiency.

In addition to this clinical study, the results of a questionnaire study on neuromuscular features, fatigue, pain, and impairments are described (*Chapter 5 and 6*). We used a multidimensional assessment method to measure fatigue, its clinical relevance, and possible determinants among 273 members of the Dutch Ehlers-Danlos syndrome patient organization. The following dimensions were assessed: fatigue severity, functional impairment in daily life, physical activity, psychological distress, sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, causal attribution of fatigue, pain, and disease related factors (*Chapter 5*). Since pain severity was found to be one of the determinants of fatigue in EDS, we subsequently investigated various aspects of pain, functional impairment due to pain, and sleep disturbances (*Chapter 6*).

Part IIB: Quantitative muscle function measurements of tenascin X-deficient Ehlers-Danlos syndrome patients and tenascin xb knockout mice

The studies described in this part focus on the pathophysiological mechanisms contributing to the neuromuscular phenotype of EDS. These studies were performed on TNX-deficient EDS patients and on *Tnxb* knockout (KO) mice, an animal model of EDS.

We first performed a pilot study on two EDS patients (*Chapter 7*), consisting of clinical investigations, electromyography, muscle ultrasound, muscle biopsy, and quantitative muscle function tests on two EDS patients with deficiency of TNX. Based on the results, we hypothesized that alterations in the ECM modify myofascial force transmission (i.e. the transmission of generated force via connective tissue structures within and between muscles, enabling them to work together) and consequently influence muscle function in EDS.

To test this hypothesis, we subsequently performed an extensive quantitative muscle function study on *Tnxb* KO mice (*Chapter 9*). We first performed a study on the muscular phenotype of these *Tnxb* KO mice (*Chapter 8*). This consisted of standardized clinical assessment, muscle histology, and gene expression profiling of muscle tissue. Furthermore, peripheral nerve composition was studied by histology and electronmicroscopy. The quantitative muscle function study protocol (*Chapter 9*) focused on both intra- and intermuscular aspects of muscle force. Intramuscular aspects were studied during isometric contractions of isolated muscles, when the muscle–tendon complex length is fixed. In this situation, the actual active length of the muscle fibres is dependent on the properties of the series elastic components, consisting of the network of endo-, peri-, and epimysium, and of the tendon. Study of the intermuscular aspects of muscle force has proved to be an effective method to investigate myofascial force transmission.[4]

To confirm these findings in EDS patients, we finally performed a quantitative muscle function measurement on seven TNX-deficient EDS patients (*Chapter 10*). This consisted of measurements of knee flexion and extension at different joint angles and of an evaluation of voluntary activation capacity.

Part III: Neuromuscular features of Marfan syndrome

Clinical evaluation of Marfan syndrome patients

In order to find out whether the clinical findings in EDS can be generalized to other ICTDs, an observational study on neuromuscular features in Marfan syndrome was performed, the results of which are described in *Chapter 11*. Neuromuscular involvement was evaluated in ten Marfan patients through a standardized questionnaire, physical examination, nerve conduction study, needle electromyography, muscle ultrasound, laboratory investigation, and muscle biopsy. Existing neuroimages were screened for dural ectasia and spinal meningeal cysts.

Since the results also showed signs of lumbosacral radiculopathy associated with dural ectasia in the elder Marfan patients, we investigated three patients with moderate to severe lumbosacral radiculopathy in more detail. This case series is presented in *Chapter 12*.

Part IV: Summary and outlook

Finally, part IV provides an overview of the results of the previously described chapters. General conclusions and directions for future research are formulated as well (*Chapter 13*).

Table 1 Schematic presentation of the outline of this thesis.

	Part I					
Literature review	General introduction and outline of this thesis **Chapter 1**					
	Literature review on the molecular and clinical overlap between inherited connective tissue disorders and myopathies **Chapter 2**					
	Part IIA					**Part III**
	EDS TNX-deficient type	*EDS hypermobility type (w/wo* TNXB *haploinsufficiency)*	*EDS Vascular type*	*EDS Classical type*	*EDS Kyphoscoliotic type*	*Marfan syndrome*
	Case report	Case report	No case report **Chapter 3**	No case report	Case report	Case series **Chapter 12**
Clinical observations and systematic studies in EDS and Marfan syndrome	History taking and physical examination Laboratory investigations Electromyography Muscle ultrasound Muscle biopsy: histology **Chapter 4**				Not included	History taking and physical examination Laboratory investigations Electromyography Muscle ultrasound Muscle biopsy: histology **Chapter 11**
	Questionnaire study **Chapter 5 and 6**					No questionnaire study on pain and fatigue

<table>
<tr><th colspan="4">Part IIB</th></tr>
<tr><td></td><td>EDS
TNX-deficient type</td><td>EDS hypermobility type
(reduced TNX serum level)</td><td>Tnxb KO mice</td></tr>
<tr><td rowspan="2">Quantitative muscle function measurements in TNX-deficient EDS and Tnxb KO mice</td><td colspan="2">Pilot study of quantitative muscle testing
Chapter 7</td><td>Muscle function evaluation and muscle biopsy
Chapter 8</td></tr>
<tr><td>Quantitative muscle function measurements
Chapter 10</td><td>Not included</td><td>Quantitative muscle function measurements
Chapter 9</td></tr>
<tr><th colspan="4">Part IV</th></tr>
<tr><td colspan="4">Summary, general discussion, and directions for further research
Chapter 13</td></tr>
</table>

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Loeys BL, Dietz HC, Braverman AC, Callewaert BL, De BJ, Devereux RB, Hilhorst-Hofstee Y, Jondeau G, Faivre L, Milewicz DM, Pyeritz RE, Sponseller PD, Wordsworth P, De Paepe AM. The revised Ghent nosology for the Marfan syndrome. J Med Genet 2010; 47: 476-485.
3. Jobsis GJ, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA. Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. Nat Genet 1996; 14: 113-115.
4. Huijing PA. Epimuscular myofascial force transmission: a historical review and implications for new research. International Society of Biomechanics Muybridge Award Lecture, Taipei, 2007. J Biomech 2009; 42: 9-21.
5. Besselink-Lobanova A, Maandag NJ, Voermans NC, van der Heijden HF, van der Hoeven JG, Heunks LM. Trachea rupture in tenascin-X-deficient type Ehlers-Danlos syndrome. Anesthesiology 2010; 113: 746-749.
6. Gawthrop F, Mould R, Sperritt A, Neale F. Ehlers-Danlos syndrome. BMJ 2007; 335: 448-450.
7. Berglund B, Anne-Cathrine M, Randers I. Dignity not fully upheld when seeking health care: experiences expressed by individuals suffering from Ehlers-Danlos syndrome. Disabil Rehabil 2010; 32: 1-7.
8. Stamenkovic I. Extracellular matrix remodelling: the role of matrix metalloproteinases. J Pathol 2003; 200: 448-464.
9. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 2002; 13: 377-383.
10. Campbell KP, Stull JT. Skeletal muscle basement membrane-sarcolemma-cytoskeleton interaction minireview series. J Biol Chem 2003; 278: 12599-12600.
11. Kjaer M, Magnusson P, Krogsgaard M, Boysen MJ, Olesen J, Heinemeier K, Hansen M, Haraldsson B, Koskinen S, Esmarck B, Langberg H. Extracellular matrix adaptation of tendon and skeletal muscle to exercise. J Anat 2006; 208: 445-450.
12. Schessl J, Zou Y, Bonnemann CG. Congenital muscular dystrophies and the extracellular matrix. Semin Pediatr Neurol 2006; 13: 80-89.
13. Kaindl AM, Jakubiczka S, Lucke T, Bartsch O, Weis J, Stoltenburg-Didinger G, Aksu F, Oexle K, Koehler K, Huebner A. Homozygous microdeletion of chromosome 4q11-q12 causes severe limb-girdle muscular dystrophy type 2E with joint hyperlaxity and contractures. Hum Mutat 2005; 26: 279-280.
14. Mercuri E, Yuva Y, Brown SC, Brockington M, Kinali M, Jungbluth H, Feng L, Sewry CA, Muntoni F. Collagen VI involvement in Ullrich syndrome: a clinical, genetic, and immunohistochemical study. Neurology 2002; 58: 1354-1359.
15. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, Anton-Lamprecht I, Muntoni F, Hanefeld F, Bonnemann CG. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A 2005; 132: 296-301.
16. Lampe AK, Bushby KM. Collagen VI related muscle disorders. J Med Genet 2005; 42: 673-685.
17. Voermans NC, Bonnemann CG, Hamel BC, Jungbluth H, van Engelen BG. Joint hypermobility as a distinctive feature in the differential diagnosis of myopathies. J Neurol 2009; 256: 13-27.
18. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
19. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
20. Castori M, Camerota F, Celletti C, Danese C, Santilli V, Saraceni VM, Grammatico P. Natural history and manifestations of the hypermobility type Ehlers-Danlos syndrome: a pilot study on 21 patients. Am J Med Genet A 2010; 152A: 556-564.
21. Maas H, Sandercock TG. Force transmission between synergistic skeletal muscles through connective tissue linkages. J Biomed Biotechnol 2010; 2010: 575672.
22. Rijkelijkhuizen JM, Baan GC, de Haan A, de Ruiter CJ, Huijing PA. Extramuscular myofascial force transmission for in situ rat medial gastrocnemius and plantaris muscles in progressive stages of dissection. J Exp Biol 2005; 208: 129-140.

23. Huijing PA, Baan GC. Myofascial force transmission via extramuscular pathways occurs between antagonistic muscles. Cells Tissues Organs 2008; 188: 400-414.
24. Castori M, Camerota F, Celletti C, Grammatico P, Padua L. Quality of life in the classic and hypermobility types of Ehlers-Danlos syndrome. Ann Neurol 2010; 67: 145-146.
25. Pretorius ME, Butler IJ. Neurologic manifestations of Ehlers-Danlos syndrome. Neurology 1983; 33: 1087-1089.
26. Banerjee G, Agarwal RK, Shembesh NM, el Mauhoub M. Ehlers Danlos syndrome--masquerading as primary muscle disease. Postgrad Med J 1988; 64: 126-127.
27. Boileau C, Jondeau G, Mizuguchi T, Matsumoto N. Molecular genetics of Marfan syndrome. Curr Opin Cardiol 2005; 20: 194-200.
28. Angelin A, Tiepolo T, Sabatelli P, Grumati P, Bergamin N, Golfieri C, Mattioli E, Gualandi F, Ferlini A, Merlini L, Maraldi NM, Bonaldo P, Bernardi P. Mitochondrial dysfunction in the pathogenesis of Ullrich congenital muscular dystrophy and prospective therapy with cyclosporins. Proc Natl Acad Sci U S A 2007; 104: 991-996.
29. Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, Blaauw B, Urciuolo A, Tiepolo T, Merlini L, Maraldi NM, Bernardi P, Sandri M, Bonaldo P. Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. Nat Med 2010; 16: 1313-1320.
30. Davies KE, Nowak KJ. Molecular mechanisms of muscular dystrophies: old and new players. Nat Rev Mol Cell Biol 2006; 7: 762-773.
31. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.

CHAPTER 2

Clinical and molecular overlap between myopathies and inherited connective tissue diseases

Adapted from:
Voermans NC, Bönnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ.
Neuromuscul Disord. 2008;18:843-56.

Abstract

This review presents an overview of the myopathies and inherited connective tissue disorders that are caused by defects or deficiencies of molecules within the extracellular matrix (ECM). We will cover the myopathies caused by defects in transmembrane protein complexes (dystroglycan, sarcoglycan, and integrins), laminin, and collagens (collagen VI, XIII, and XV). Clinical characteristics of several of these myopathies imply skin or joint symptoms. We subsequently describe the inherited connective tissue disorders that are characterized by mild to moderate muscle involvement in addition to the dermal, vascular, or articular symptoms. These disorders are caused by defects of matrix-embedded ECM molecules that are also present within muscle (collagen I, III, V, IX, lysylhydroxylase, tenascin, fibrillin, fibulin, elastin, and perlecan).

By focusing on the structure and function of these ECM molecules, we aim to point out the clinical and molecular overlap between these disorders. We argue that clinicians and researchers dealing with myopathies and inherited connective tissue disorders should be aware of this overlap. Only a multi-disciplinary approach will allow a full recognition of the wide variety of symptoms present in the spectrum of ECM defects, which has important implications for scientific research, diagnosis, and for the treatment of these disorders.

Introduction

Clinical overlap between myopathies and inherited connective tissue diseases

Over the past few years, a number of patients with Ehlers-Danlos syndrome (EDS) or Marfan syndrome were referred to our neuromuscular outpatients' clinic by the departments of Dermatology and Human Genetics. These patients suffered from muscle weakness, hypotonia, exercise intolerance, and / or easy fatigability. Concomitantly, patients with similar complaints were referred for neuromuscular work-up by their general practitioner or by other neurologists. None of these patients was diagnosed with a neuromuscular disorder. However, clinical investigations raised the suspicions of an inherited connective tissue disorder (ICTD), and most of these patients were diagnosed with EDS or Marfan syndrome.

Exposure to both patient groups has sparked our interest in the clinical overlap between certain myopathies and ICTDs. Scientific progress in various fields has contributed to the understanding of this overlap. First, biochemical research provides a growing insight in the structure and function of various extracellular matrix (ECM) molecules of skeletal muscle.[1] Furthermore, growth factors and cytokines are increasingly recognized as players in the field of muscular pathophysiology.[2-4] Second, focus in neuromuscular research has gradually extended from the cytoskeleton and the sarcolemma towards the ECM.[5] This results in an increased recognition of myopathies that result from defects in ECM molecules (e.g. Bethlem myopathy and Ullrich Congenital Muscular Dystrophy (UCMD)).[6] Clinical characteristics of these myopathies also include skin and joint features, as seen in the ICTDs.[7] Third, clinical research in the field of ICTDs has renewed interest for myopathic features.[8-10] This has shed light on the roles of ECM molecules in pathophysiology and towards possible interventions.[4]

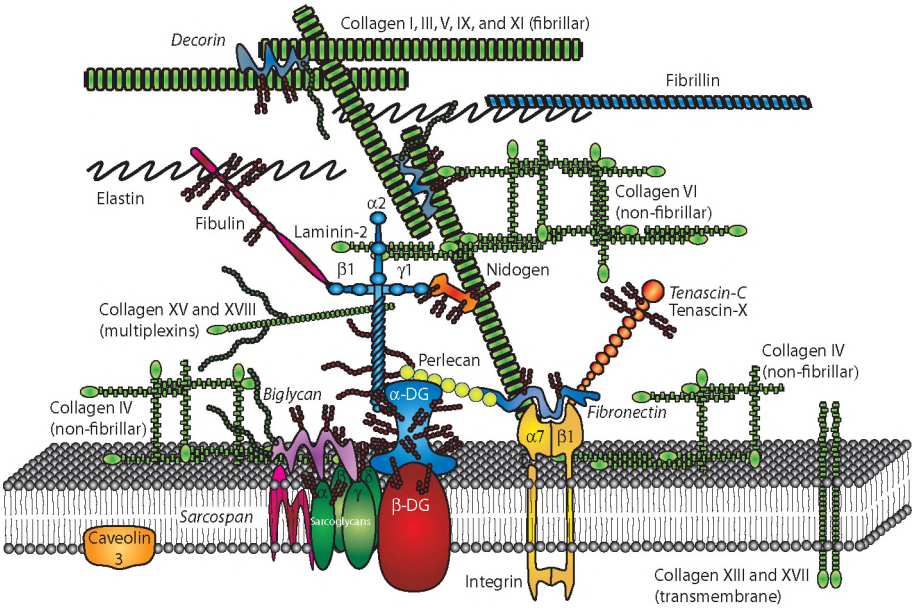
With this review, we aim to provide an overview of the myopathies and ICTDs that result from defective ECM molecules. A general awareness of the overlap in clinical features between these pathologies may have important implications for research, diagnosis, and therapy (for an overview of ECM molecules that are implicated in myopathies or ICTDs, see *Table 2* and *Figure 1*). Although certain clinical characteristics seen in the conditions under discussion also occur in disorders caused by defects of other molecules (e.g. contractures in Emery-Dreifuss Muscular Dystrophy caused by lamin or emerin mutations), this review will only focus on primary ECM defects. Nevertheless, future research into a possible pathophysiological role of the ECM in these myopathies would be very interesting. Furthermore, we will not discuss the disorders of the neuromuscular junction, although some of these are also caused by defects of ECM molecules.

Extracellular matrix and connective tissue

The ECM of skeletal muscle refers to the extracellular network, which consists of non-collagenous glycoproteins and fibrous proteins (*Figure 1*). This network can be compared to

Figure 1 Schematic representation of the ECM surrounding skeletal muscle.

Individual molecules are depicted at their approximate location in relation to the sarcolemma. Well-established molecular interactions between individual ECM molecules are portrayed. Due to limitations in space, not all possible interactions are indicated. Names of ECM molecules of which no genetic mutations are associated with specific myopathies or inherited connective tissue disorders in human, are printed in Italic (DG = dystroglycan).



composite plastics or reinforced concrete: the amorphous matrix of non-collagenous glycoproteins and proteoglycans (formerly referred to as 'ground substance') is reinforced by stiffer fibrous proteins. This matrix yields a supramolecular network that can both withstand and transmit the contractile forces generated by muscle fibres. Most likely, the proteins and glycoproteins of the sarcolemma and the ECM most proximal to muscle fibres are predominantly produced by muscle cells. The other proteins and glycoproteins of the ECM are assumed to be produced by a combination of muscle cells and interstitial cells, including fibroblasts and/or myofibroblasts; however, much research needs to be done in this field.[11] The ECM provides intramuscular continuations of neurovascular tracts in which nerve branches and blood vessels are embedded (*Figure 2*).[12] This integral network not only ensures the structural integrity and enables force transmission onto the skeleton; it also mediates the development and physiological behaviour of muscle cells.

The ECM has long been viewed as an amorphous scaffold that provides mechanical support.[13] Recent insights underscore the importance of the ECM in developmental and regenerative processes, as well as in transmembrane signalling and force transmission.[2,14] In fact, the ECM is a very dynamic structure that easily adapts to changes in physiological demand. Constant remodelling modifies the mechanical and viscoelastic properties, decreases stress-susceptibility, and may increase load-resistance.[13,15]

Classification of components of muscle ECM is based upon histological features (light microscopy), ultrastructural features (electron microscopy), or on molecular features. These classifications are overlapping to a certain extent (*Table 1*). Connective tissue in skeletal muscle is histologically defined as the ECM with the nerve branches, capillaries, fibroblasts, and macrophages that are embedded within this matrix. The following structures within muscle connective tissue are recognized: (1) endomysium surrounding each muscle fibre, (2) perimysium surrounding groups of muscle fibres (so called fascicles), and (3) epimysium surrounding the muscle as a whole. The endo-, peri-, and epimysium form an intricate supporting network, which integrates muscular forces and connects muscle fibres to the tendon, which itself is attached to the bone (*Figure 2*). Myotendinous junctions are complex specializations of the sarcolemma and ECM that connect the ends of muscle fibres to tendons or aponeuroses.[13] Ultrastructurally, the ECM of skeletal muscle adjacent to muscle cells is designated basement membrane. The basement membrane is composed of two layers: the basal lamina lies proximal to the sarcolemma, and the reticular lamina lies more distally. This is surrounded by more peripherally located ECM. A third classification can be made at the molecular level: ECM molecules that are directly associated with the sarcolemma ('sarcolemma-associated ECM molecules') versus ECM molecules that are embedded within the collagen-reinforced matrix ('matrix-embedded ECM molecules').

In this review, we will focus on the structural proteins and glycoproteins of the ECM that are involved in muscular symptoms in humans or in animal models. Although equally important in muscle physiology and pathology, we will not discuss the more dynamic molecules that are involved in various muscle diseases (e.g. growth factors, cytokines, and matrix metalloproteinases).

Myopathies: from the sarcolemma towards the extracellular matrix

The major focus in neuromuscular research has long been the myofibrils, the cytoskeleton, and the cell membrane. Disorders of the contractile elements mostly belong to the congenital myopathies, whereas defects of the cell membrane and its connection to the cytoskeleton are associated with the muscular dystrophies.[16,17]

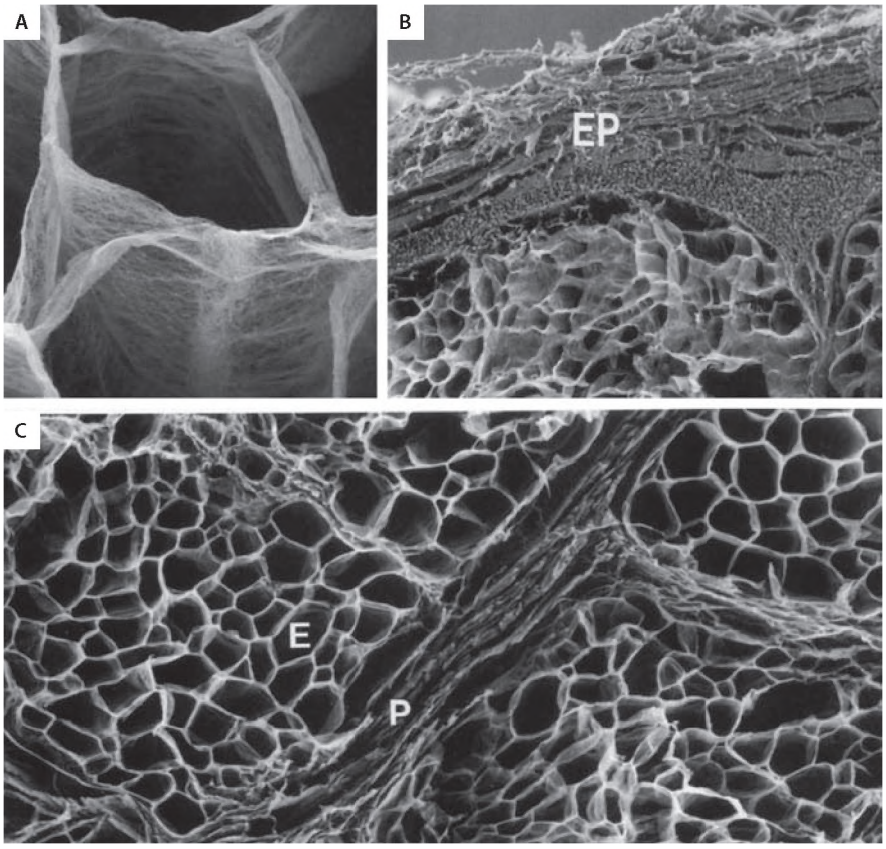
Table 1 Definitions.

Histological classification
Connective tissue: matrix of extracellular material, consisting of the ECM and of the nerve branches, capillaries, fibroblasts, and macrophages that are embedded within this matrix (Figure 2)
Endomysium: the collagen reinforced ECM that surrounds individual muscle cells
Perimysium: the collagen reinforced ECM that surrounds fascicles of muscle cells
Epimysium: the collagen reinforced ECM that surrounds entire muscles
Ultrastructural classification
Sarcolemma: the plasma membrane of a muscle cell, with basal lamina receptors dystroglycan and sarcoglycan
Extracellular matrix (ECM): is the supramolecular, extracellular network that consists of non-collagenous proteoglycans and glycoproteins and is reinforced by stiffer collagenous proteins. The ECM includes the extracellular parts of transmembrane molecules, which protrude in the ECM and interact with other ECM molecules
Basement membrane: the ECM surrounding skeletal muscle cells. It is comprised of two layers: the inner basal lamina and the outer reticular lamina
Basal lamina: the ECM that is directly adjacent to the sarcolemma, which consists of the molecules that interact with the trans-sarcolemmal molecules
Reticular lamina: the ECM zone that is located adjacent to the basal lamina, which consists of various glycoproteins and collagens
More peripheral ECM that is found peripherally of the basement membrane
Molecular classification
Sarcolemma-associated ECM molecules: ECM molecules that are located at or directly adjacent to the sarcolemma; including dystroglycan, sarcoglycan, integrins and their directly associated molecules (located in the basal lamina); they are part of the chain spanning the sarcolemma and enabling force transmission (Figure 1)
Matrix-embedded ECM molecules: the ECM molecules that are located more peripherally in the matrix and interact with each other (located in the reticular lamina and more peripherally in ECM)

In recent years, the focus of attention has gradually shifted towards the ECM.[5] This was initiated by the growing insight that the ECM is an extremely dynamic complex of molecules that interacts with sarcolemmal, cytoskeletal, and nuclear elements in order to maintain skeletal muscle integrity and to transmit forces.[18] Since then, myopathies have been identified that result from defects in or deficiencies of trans-sarcolemmal proteins (e.g. limb girdle muscular dystrophies) or ECM molecules (e.g. congenital muscular dystrophies, Bethlem myopathy and UCMD). Extramuscular characteristics of such myopathies, including joint and skin manifestations, are increasingly recognized.[7,19]

Figure 2 Structure of intramuscular connective tissue.

The skeletal muscle (bovine semitendinosus muscle) extracellular network is shown by scanning electron micrographs after removal of skeletal muscle fibres. **A:** The endomysium surrounding one individual skeletal muscle fibre. **B:** The epimysium (EP) surrounding the entire muscle. **C:** The perimysium (P), surrounding a fascicle, as well as the endomysium (E). Reprinted with permission from S. Karger AG, Basel.[12]



Sarcolemma-associated extracellular matrix molecules and myopathies

The trans-sarcolemmal molecules dystroglycan, sarcoglycan, and the associated molecule integrins are part of the complex of proteins that connects muscle cells to the surrounding ECM. Dystroglycans and sarcoglycans, together with dystrophin, form the dystrophin-glycoprotein complex (DGC). This complex has both mechanical and signalling roles in mediating interactions between the cytoskeleton, the sarcolemma, and the ECM. The proteins within the DGC are structurally organized into three distinct subcomplexes. These are the (1)

intracellular proteins dystrophin, α-dystrobrevin, and the syntrophins (which will not be discussed in this review; we refer to a recent review by Laval et al);[20] (2) the sarcolemmal dystroglycan (β subunit); and (3) the sarcoglycan-subcomplex (α, β, γ, and δ subunit) (*Figure 1*). A second group of sarcolemma-associated ECM molecules forming a link to the more peripheral ECM are the integrins and α-dystroglycan (*Figure 1*). Integrins are a large family of transmembrane proteins that mediate cell-matrix as well as cell-cell recognition and adhesion.

Both DGC and integrin complexes are localized in the sarcolemma and protrude into the ECM to protect the integrity of the sarcolemma and associated structures against physical damage during repeated rounds of contraction and relaxation. These complexes not only form a physical link between the cytoskeleton and the basal lamina, they are also instrumental in the mechanical force transmission and biochemical transmembrane signalling.[21,22] Disruption of these complexes leads to muscular dystrophy.[20,23]

α-Dystroglycan and defects of O-mannosyl-glycosylation

Dystroglycan is composed of tightly associated subunits α and β, which are encoded by a single gene (*DAG1*), and cleaved and glycosylated post-translationally. β-Dystroglycan spans the sarcolemma, which enables intracellular binding to dystrophin, which itself is connected to the actin cytoskeleton (*Figure 1*). Extracellularly, β-dystroglycan binds to α-dystroglycan, which consists of a core protein (74 kD) that is heavily and variably glycosylated, resulting in forms with apparent molecular weights as high as 200 kDa. O-linked glycosylation is the most important contributor to this modification, which is important for many ligand-receptor interactions.[24]

Glycosylated α-dystroglycan in muscle binds to the ECM proteins laminin α2, biglycan, and perlecan (*Figure 1*). The dystroglycan complex has an important receptor function and may participate in formation and maintenance of the basement membrane by promoting the polymerization of laminin on the cell surface.[25]

Mutations in the dystroglycan gene have not been described in humans; most likely, such mutations are lethal in an early stage. In mice, systemic knock-out models are lethal in an early embryonal stage; selective depletion of dystroglycan in mature muscle by chimeric expression or tissue specific knock-out mice causes a progressive myopathy with abnormal localization of basal lamina components laminin and collagen IV, and abnormalities of the neuromuscular junctions.[25] However, defects in α-dystroglycan O-mannosyl glycosylation results in several forms of congenital muscular dystrophy (MDC) as well as limb-girdle muscular dystrophy (LGMD). The aberrant O-glycosylation is caused by mutations in proven or putative glycosyltransferase that participate in this process. These MDCs are therefore referred to as secondary dystroglycanopathies. Hypoglycosylation of dystroglycan impairs its ligand binding, which causes disruption of the link between the cytoskeleton and the ECM, probably contributing to the development of muscular dystrophy.[24]

So far, six genes have been identified to be involved in the O-mannosyl glycosylation of α-dystroglycan, mutations of which result in myopathies and abnormalities neuronal migration. These disorders manifest a spectrum that includes Muscle-eye-brain disease (MEB), Walker-Warburg syndrome (WWS), Fukuyama-type congenital muscular dystrophy (FCMD), congenital muscular dystrophy type 1C and 1D (MDC 1C and 1D), and limb girdle muscular dystrophy 2I, 2K, and 2M (LGMD 2I, 2K, and 2M).[5,17] Since O-mannosyl glycosylation of α-dystroglycan also occurs in the central nervous system and in the eye, these myopathies are associated with brain and eye abnormalities, in particular with lissencephaly type II (cobblestone complex) and with pontocerebellar changes. Eye abnormalities include both anterior and posterior anomalies such as cataract, microcornea and microphthalmia, lens defects, retinal detachment and dysplasia, optic nerve and macula hypoplasia and atrophy. Although α-dystroglycan was also shown to be present in the epidermis in skin and produced by both keratinocytes and fibroblasts in vitro, neither dermal nor articular symptoms have been described in patients with α-dystroglycanopathies so far.[26]

Laminin α2 is associated with the dystrophin–glycoprotein complex. Whereas it is not a part of this complex, it is the major extracellular binding partner for α-dystroglycan thus helping to anchor the complex in the ECM. A secondary deficiency of laminin α2 occurs in some MDC syndromes associated with defective α-dystroglycan O-mannosyl glycosylation, including MDC1B, MEB disease, and Fukuyama MDC.[27] Increasing insight in the molecular pathogenesis of the α-dystroglycanopathies has sparked some new therapeutic options in this group of disorders. Illustrative to this end is the putative human glycosyltransferase LARGE, which acts as a facilitator in the process of α-dystroglycan glycosylation. Overexpression of *LARGE* improves α-dystroglycan glycosylation in a mouse model deficient in LARGE, as well as in other α-dystroglycanopathy mouse models, thus opening perspectives for clinical intervention.[28]

Sarcoglycan and sarcoglycanopathies (LGMD 2C-F)

Sarcoglycans are N-glycosylated transmembrane proteins with a short intra-cellular domain, a single transmembrane region, and a large extra-cellular domain containing a group of conserved cysteine residues. Sarcoglycans thus form a membrane-spanning complex of glycoproteins, which is closely associated with the dystroglycan complex. Similar to dystroglycan, the sarcoglycan complex is located at the basal lamina (*Figure 1*). So far, six sarcoglycan proteins have been identified (α, β, γ, δ, ε, and φ), ranging in size from 35-50 kDa. The major sarcoglycan complex in skeletal muscle is mainly composed of α, β, γ, and δ sarcoglycan. The sarcoglycan complex is formed in the endoplasmic reticulum. It associates with the dystroglycan complex and sarcospan en route from the Golgi apparatus to the cell surface.[29] Whereas the dystroglycan-complex is found in nearly all cell types, the α, β, γ, δ sarcoglycan complex is primarily found in muscle.[30]

In skeletal muscle, the sarcoglycan complex has a number of putative functions. Intracellularly, the sarcoglycan complex binds γ-filamin, which is a major muscle architectural protein located in the Z-line under the sarcolemma that binds actin. It is a component of the chain binding the sarcolemma to the sarcomeric protein complex and as such likely involved in signal transduction and force transmission. Extracellularly, the sarcoglycan complex binds to biglycan and is associated with α-dystroglycan (either directly or through biglycan).[31] Sarcospan probably contributes to the stabilisation of components of the dystrophin-glycoprotein complex as alpha dystrophin-glycoprotein localization is lost in mutations of the sarcoglycan complex.[20]

Mutations in genes encoding the sarcoglycans cause a number autosomal-recessive limb-girdle muscular dystrophies (sarcoglycanopathies; LGMD 2C-F): LGMD 2D (α- sarcoglycan), LGMD 2E (β- sarcoglycan), LGMD 2C (γ-sarcoglycan), and LGMD 2F (δ-sarcoglycan). Their clinical presentations range from severe forms with rapid onset and progression to very mild forms that allow patients to have a fairly normal lifespan and activity levels. We refer to a recent review for an extensive description of the clinical characteristics.[20] The sarcoglycan complex is primarily expressed in muscle, and dermal or articular features have not been described. Secondary skeletal deformities such as hyperlordosis and scoliosis can occur (*Table 2*).

α7β1 Integrin and congenital myopathy

Integrins are sarcolemma-spanning proteins of which the extracellular domain extends into the ECM. Integrins contain large (α) and small (β) subunits of 120-170 kDa and 90-100 kDa in size, respectively (*Figure 1*), and have binding sites for divalent cations (Mg^{2+} and Ca^{2+}), which are necessary for their adhesive function. There are many integrin isoforms, and many cells express multiple isoforms on their surface. The expression of α7 integrin (*ITGA7*) in myocytes is developmentally regulated. In adult muscle, mainly α7β1 integrin persists, which is concentrated in myotendinous junctions but also present in neuromuscular junctions and along the sarcolemmal membrane. α7β1 Integrin provides resistance to exercise-induced muscle damage.[32] Recent animal studies indicate complementary roles for the dystrophin and the dystrophin associated glycoprotein complex and α7β1 integrin in maintaining the functional integrity of skeletal muscle.[33] In myotendinous junctions, α7β1 integrin organizes and maintains the connection between muscle and tendon and also functions as a laminin receptor.[34] α7β1 Integrin is also expressed in the peripheral nervous system, where it probably functions as a Schwann cell receptor for laminin-2, providing a transmembrane linkage between the ECM and the cytoskeleton.[35] Furthermore, α7β1 integrin plays an important role in vascular development and integrity.[36]

Because of their transmembrane structure and association with other molecules, integrins mediate mechanical and functional continuity between the inside and the outside

of the cell, as well as transmembrane signalling. The cytoplasmic domain of integrin binds to cytoskeleton proteins like talin, vinculin, and α-actinin (which will not be discussed in this review). Extracellularly, α7β1 integrin acts as a laminin receptor; it may interact with laminin, collagen IV, and fibronectin, the latter of which links to tenascin-X (TNX) (*Figure 1*). Integrin signalling is bidirectional. 'Inside-out' signals regulate integrin affinity for adhesive ligands, and ligand-dependent 'outside-in' signals regulate cellular responses to adhesion.[34]

Targeted deletion of the α7β1 integrin gene leads to a mild form of muscular dystrophy in mice.[37] Histological analysis of integrin null skeletal muscle revealed typical symptoms of a progressive muscular dystrophy starting soon after birth in mice. It also shows impaired myotendinous binding due to a decrease of interdigitations, and the retraction of myofilaments from the sarcolemmal membrane.[37] Subsequent studies revealed mutations of the α7 integrin gene in three patients with congenital myopathy; one of these patients was mentally retarded, and one of them had a congenital hip dislocation. No skin or other joint features were reported (*Table 2*).[38] No additional patients have been reported since this initial report, suggesting that this is a very rare myopathy. Since integrins provide a major link between the cytoskeleton and the ECM, the genes encoding integrins may be candidate genes for other, as yet unclassified muscular dystrophies and myopathies.[34]

Laminin and congenital muscular dystrophy

Laminin is an omnipresent component of developing and adult muscle basal lamina. Laminin is composed of three distinct subunits (α-, β-, and γ-chain; 400, 210, and 200 kDa, respectively), which oligomerize to form a cruciform protein (*Figure 1*). Five genes are known for the α-chain, three for the β-chain, and three for the γ-chain. Eleven distinct laminin isoforms are synthesized by a wide variety of cells in a tissue-specific manner. The extrasynaptic basement membrane of adult muscle is rich in laminin-2 (merosin), whereas the synaptic basement membrane contains laminin-4, laminin-9, and laminin-11.

Laminins modulate a variety of cellular activities, including cell-cell recognition, differentiation, cell survival, and transmission of force.[39] Laminins interact with a number of ECM macromolecules such as nidogen, agrin, perlecan, and collagen IV. This laminin-containing network is tightly connected to the sarcolemma through two major transmembrane laminin receptors: the dystrophin-glycoprotein complex and the integrins (*Figure 1*). Laminin-α2 is also expressed in the central and peripheral nervous system, in sensory nerves, in skin, at the epidermal/dermal junction, and around hair follicles.

Primary or secondary laminin α2 deficiency causes various forms of congenital muscular dystrophy (MDC 1A, 1B, and 1C), in which impaired anchoring of muscle cells in the ECM results in impaired membrane stability and increased muscle fibre apoptosis.[40] Histologically, this results in muscular dystrophy with muscle fibre necrosis and regeneration, combined with endo- and perimysial fibrosis and inflammation. MDCs may be accompanied by mental

Table 2 Overview of the ECM molecules that are implicated in myopathies or inherited connective tissue disorders.

Molecule	Tissue distribution	Disease or mouse model
Sarcolemma-associated ECM molecules (basal lamina of basement membrane)		
α-Dystroglycan glycosylation	muscle, skin, CNS	MEB
		WWS
		FCMD
		MDC 1C
		MDC 1D
		LGMD2I
		LGMD2K
		LGMD 2M
β-Dystroglycan	muscle, skin, CNS	Mouse model: lethal
α-Sarcoglycan	muscle	LGMD 2D
β-Sarcoglycan	muscle	LGMD 2E
γ-Sarcoglycan	muscle	LGMD 2C
δ-Sarcoglycan	muscle	LGMD 2F
α7β1 Integrin	muscle, PNS	Integrin α7 deficiency
Laminin α2	muscle, skin, CNS, PNS	MDC 1A, 1B and 1C
Collagen VI	muscle, epithelium, cornea	UCMD
		Bethlem myopathy
Collagen XIII	muscle	Mouse model: myopathy
Collagen XV	muscle, vessels	Mouse model: myopathy
Matrix-embedded ECM molecules (reticular lamina of basement membrane and more peripheral ECM)		
Collagen I	throughout body, except cartilage	Osteogenesis imperfecta
		EDS arthrochalasia type
Collagen III	muscle, skin, PNS vessels, GI	EDS vascular type
Collagen V	throughout body, except cartilage	EDS classical type
Collagen IX	predominantly cartilage	MED with myopathy
		Mouse model: reduced muscle
Tenascin-C	muscle, skin, CNS, GI	strength
Tenascin-X	muscle, skin, PNS, tendon, vessels	EDS TNX-deficient type
Fibrillin-1	muscle, skin, bones, eye	Marfan syndrome
Glycosylation of fibulin	muscle, skin	Cutis Laxa
Elastin	muscle, skin	Cutis Laxa
Perlecan	muscle, bones	SJS, SHS

Indicated are the distribution among various tissues, associated disease or available mouse models, major symptoms, and gene locus. Molecules are subdivided as sarcolemma-associated or matrix-embedded. Abbreviations: CNS: central nervous system; GI: gastrointestinal system; M, muscular symptoms; D, dermal symptoms; A, articular symptoms; S, skeletal symptoms; +, present; -, absent or not reported.

Gene (gene locus)	Location of symptoms					
	M	D	A	S	CNS	Other
POMGnT1 (1q3); *POMT1* (9q34)	+	-	-	-	+	Ocular
POMT1 (9q34); *POMT2; FKRP; FKTN*	+	-	-	-	+	Ocular
FKTN (9q3)	+	-	+	-	+	Ocular
FKRP (19q13.3)	+	-	+	-	+	
LARGE (22q12.3-q13.1)	+	-	+	-	+	
FKRP (19)	+	-	+	-	-	
POMT1 (9q34)	+	-	+	-	+	
FKTN (9q3)	+	-	-	-	-	
3q21	+	-	-	-	-	
SGCA (17q21)	+	-	-	-	-	
SGCB (4q12)	+	-	-	-	-	
SGCC (13q12)	+	-	-	+	-	Hearing loss
SGCD (5q33)	+	-	-	-	-	
ITGA7 (12q13)	+	-	+	-	+	
LAMA (6q2)	+	-	+	-	+	
COL6A1 (21q), *COL6A2* (21q2) and *COL6A3* (2q37)	+	+	+	+	-	
COL6A1 (21q22.3), *COL6A2* (21q2) and *COL6A3* (2q37)	+	+	+	-	-	
COL13A1 (10q22)	+	-	-	-	-	
COL15A1 (9q21-q22)	+	-	-	-	-	
COL1A1 (17q21.31-q22); *COL1A2* (7q22.1)	+	+	+	+	-	Ocular, Ear
COL1A1 (17q21.31-q22); *COL1A2* (7q22.1)	+	+	+	+	-	
COL3A1 (2q31-q32)	+	+	+	-	-	Vascular
COL5A1 (9q34.2-q34.3) and *COL5A2* (2q31)	+	+	+	-	-	
COL9A3 (6q13)	+	-	-	+	-	
TNC (9q33)	+	+	-	-	+	
TNXB (6p21.3)	+	+	+	-	-	
FBN1 (15q21)	+	+	+	-	-	Ocular
abnormal N- and O-linked glycosylation	+	+	+	+	+	
ELN (7q11.2)	+	+	-	+	-	
HSPG2 (1p36.1)	+	-	-	+	+	

retardation (~6%), seizures (~30%), subclinical cardiac involvement, and neuronal migration defects in a small proportion of patients (*Table 2*). Although normal skin also expresses laminin α2 in the basement membrane at the junction of the dermis and epidermis, no dermal features have been reported in MDCs so far.

Mice knockout studies have shown that loss of the laminin α2 chain can be partially compensated by forced increased expression of a laminin α1 chain transgene, which is normally not expressed in muscle.[41] Most likely, laminin α1 chain in part ameliorates the development of laminin α2 chain deficient muscular dystrophy through its binding sites for α7β1 integrin and α-dystroglycan, which partially restores anchoring of muscle cells in the ECM.[41]

Matrix-embedded extracellular matrix molecules and myopathies

Collagens are ubiquitous structural proteins responsible for maintaining the mechanical integrity of virtually all tissues, but different isoforms have a tissue specific distribution. In skeletal muscle, collagens are expressed principally by fibroblasts and by myofibroblasts in the ECM. Collagen biosynthesis is a multistep process that is characterized by a large number of co- and post-translational modifications, many of them unique to collagens or collagen-like proteins. Currently, more than 20 distinct collagen isoforms have been identified. They can be classified as either non-fibrillar (e.g. IV, VI, VIII, X, and XIII) or fibrillar (e.g. I, II, III, V, XI). Some non-fibrillar collagens are transmembrane proteins (e.g. collagen XIII), whereas others form networks (e.g. collagen IV). Fibrillar collagens typically consist of three polypeptide chains wound together to form triple-helical structures, which predominantly occur in tissues that have to resist shear, tensile stress, or pressure, such as muscle, bone, cartilage, and skin.[1] *Table 3* presents an overview of the various collagen types, their distribution, function, and the related disorders.

Collagen VI, Bethlem myopathy and Ullrich congenital muscular dystrophy

The major collagen of the basal lamina is type IV collagen, whereas collagen type VI occurs in both the basal lamina and the reticular lamina. It there forms a microfibrillar network that likely interacts with collagen I, collagen IV, and with a variety of proteoglycans such as biglycan and decorin (*Figure 1*).[11] It consists of 2 different 140-kD subunits (alpha-1 and alpha-2) and a 200-kD subunit (alpha-3), and forms 150 nm long fibrils (hence, it has been called 'short-chain' collagen) (*Figure 1*). Collagen VI fibrils form a microfibrillar network.

Mutations in the gene encoding collagen VI (*COL6A*) affect collagen VI microfibril formation, which results in a disengagement of collagen VI with the basal lamina, leading to myopathy.[42] The exact mechanism and the downstream effects of collagen VI deficiency on muscle cells may involve the engagement of myofibre apoptosis, but are still subject of research.[43] Two genetically and clinically overlapping myopathies result from collagen VI deficiency: Bethlem myopathy and Ullrich congenital muscular dystrophy (UCMD). These

collagen VI myopathies may be differentiated by the age of onset, severity of muscle weakness, presence of long finger flexion contractures at onset (Bethlem myopathy), distal hyperlaxity (UCMD), presence of scoliosis, spinal rigidity, and respiratory failure (UCMD).

Bethlem myopathy is a disorder characterized by slowly progressive axial and predominantly proximal muscle weakness with flexion contractures of fingers and other joints. The disease presents with variable onset (from prenatal onset to onset in mid adulthood) and is usually only slowly progressive. The mode of inheritance documented so far is autosomal dominant, and mutations can affect any of the three *COL6A* genes. Muscle biopsy reveals normal or mildly reduced expression of collagen VI in the endomysium of most patients and loss of connection to the basement membrane. Skin features may be present in some patients and include follicular hyperkeratosis and keloid formation or "cigarette paper" scarring (*Table 2*).[6,7]

UCMD is a severe congenital myopathy including progressive atrophy and weakness of axial and appendicular muscles, coexisting with progressive contractures as well as distal joint hyperlaxity. Symptoms are usually present at birth or in early childhood. Involvement of the diaphragm can be prominent, with a varying degree of respiratory failure. Muscle biopsies from UCMD patients usually show a marked decrease or complete absence of collagen VI proximal to the sarcolemma, bolstering the hypothesis that Ullrich disease results from the loss of signalling or mechanical anchoring of the basal lamina to the interstitium. UCMD is classically regarded as an autosomal recessive disease, but many cases have now been described with de novo dominant mutations.[42] Homozygous or compound heterozygous null mutations in *COL6A* generally have a severe UCMD phenotype. In case of alternative splicing, these mutations may occasionally present a milder Bethlem myopathy-like disease. Dominant negative mutations may be as severe as the recessive null mutations.[44]

Since collagen VI distribution is not limited to muscle, extramuscular symptoms also occur. These include a striking joint hypermobility, progressive contractures, and skin abnormalities such as dry skin, protruding bulbs of hair follicles, and abnormal scar formation *(Table 2)*.[7,45] The connective tissue features were already recognized in the initial descriptions by Ullrich, and referred to in the nomenclature: 'congenital atonic sclerotic muscle dystrophy'.[46] These features are reminiscent of EDS, a ICTD that reveals clinical overlap with Bethlem myopathy.[10]

Collagen XIII and mouse model

Type XIII collagen is a transmembrane protein found at many sites of cell adhesion in various tissues. It plays an important role in the embedding of muscle fibres within the basal lamina. (*Figure 1*).[47] It is also involved in a range of integrin-mediated adherens junctions including the myotendinous junctions, costameres of skeletal muscle, and the intercalated discs in the heart.[48]

Table 3 Overview of the most currently known collagen types, their distribution, function, and the related disorders.

	Type	Distribution / function	Disorders
		Fibrillar collagens	
Fibrillar collagens: collagens that have the ability to self assemble into fibrils	I	Most abundant collagen type of the human body; present in all tissues except cartilage; major component of scar tissue, tendons, endomysium, and of epiphysis of bone.	OI EDS, arthrochalasia type
	II	Hyaline cartilage; makes up to 50% of all cartilage protein.	Stickler syndrome type I
	III	Major collagen of granulation tissue; produced by young fibroblasts before type I collagen is synthesized; also found in muscle, artery walls, intestines, and in uterus.	EDS, vascular type
	V	Co-localizes with type I collagen.	EDS, classical type
	XI	Minor component of cartilage; co-localizes with type II collagen.	Stickler syndrome type II and III
		Non-fibrillar collagens	
Network forming collagens: collagens that have the ability to form a network	IV	Present in basal lamina of eye lens, muscle, and skin, serves also as part of the filtration system in capillaries and in glomeruli in kidney.	Alport syndrome
	VIII	Present in endothelial cells, vascular smooth muscle cells	-
	X	Present in hypertrophic and mineralizing cartilage.	Metaphyseal chondroplasia
Association collagens: collagens that have the ability to associate with fibrils (Type IX, XII, XIV, XVI, XIX are FACIT's: Fibril Associated Collagens with Interrupted Triple helices)	VI	Present in most interstitial tissue; interacts with type I and IV collagen.	UCMD and Bethlem myopathy
	VII	Forms anchoring fibrils in dermal and epidermal junctions.	Epidermolysis bullosa
	IX	Occurs in cartilage; associates with type II collagen during cartilage development.	Multiple epiphyseal dysplasia AR Stickler syndrome
	XII	Interacts with collagen I fibrils, decorin, and glycosaminoglycans; is thought to act as a crossbridge between fibrils and resist shear forces.	-
	XIV	Occurs in soft connective tissues, and is associated with mature collagen fibrils.	-
	XIX	Transient expression by differentiating muscle cells.	-

Transmembrane collagens	XIII	Is transmembrane collagen; interacts with integrin, fibronectin, nidogen, and perlecan.	-
	XVII	Is transmembrane collagen.	Bullous Pemphigoid Junctional epidermolysis bullosa
Multiplexins	XV	Has adhesion function in skeletal and cardiac muscle.	-
	XVIII	Occurs predominantly in liver, lung, and kidney.	Various malignancies

Studies in mice have shown that type XIII collagen participates in the linkage between muscle fibre and basal lamina, a function which is impaired by lack of the cytosolic and transmembrane domains of this collagen. In wild-type muscle, type XIII collagen was detected in the sarcolemma, whereas truncated protein was located in the adjacent ECM. Affected skeletal muscles showed abnormal myofibres with a vague sarcolemma-ECM interphase along the muscle fibre and at the myotendinous junctions, disorganized myofilaments, and streaming of z-disks. The phenotype was progressive and aggravated by exercise.[47] No human disease has yet been associated with collagen XIII deficiency.

Collagen XV and mouse model

Collagen type XV and XVIII are structurally different from other known collagen types and belong to a subfamily of ECM proteins named multiplexins. Although these two collagens are closely related and share similarities in tissue expression, their biological roles are essentially separate. Type XV collagen is a chondroitin sulphate proteoglycan that belongs to the heterogeneous group of non-fibril-forming collagens. It is predominantly expressed in heart and in skeletal muscle and is probably involved in maintenance of the structural integrity of the ECM (*Figure 1*).[49] Type XVIII collagen was identified as a heparan sulphate proteoglycan and interacts with heparan sulfate, laminin, and perlecan.[50] It plays an important role in the regulation of muscle development and regeneration.[51]

Col15A1-deficient mice develop and reproduce normally, and are indistinguishable from their wild type littermates. However, these knockout mice show progressive focal areas of degeneration, regeneration, and variation in fibre size after 3 months of age, and they are more vulnerable to exercise-induced muscle injury than controls.[52] No human disease has yet been associated with collagen XV deficiency.

Inherited connective tissue disorders: (re)new(ed) attention for myopathic features

ICTDs result from defects in ECM molecules like collagens, fibrillin, elastin, and TNX. Although clinical symptoms are most prominent in skin, joints, and blood vessels, muscle symptoms are increasingly acknowledged, notably in Marfan syndrome, EDS, and cutis laxa.[4,8,53,54]

Matrix-embedded extracellular matrix molecules and inherited connective tissue disorders

Collagen I and III, Osteogenesis imperfecta and Ehlers-Danlos syndrome

Collagen type I and III are major components of muscle ECM that form fibrils of similar structure (300 nm, 67 nm fibrils). Type I collagen occurs throughout the body, except in

cartilage. It is the principal collagen of the dermis, fasciae, and tendons, and is a major component of mature scar tissue. Type III collagen co-polymerizes with type I collagen and is a major component of the wall of blood vessels and of hollow intestinal organs. Both types are present in epi- and peri-, as well as in endomysium. Ultrastructurally, collagen I and III are predominantly located at the reticular lamina and beyond (*Figure 1*).[1]

A defect in or deficiency of collagen I causes osteogenesis imperfecta (OI), of which four distinct collagen I-related types are described.[55] Besides brittle bones, the clinical characteristics of OI subtypes are variable and include muscle weakness, exercise intolerance, hearing loss, and fatigue (*Table 2*).[55,56] Although clinically well defined, the pathophysiology of muscle weakness in OI has not been studied yet.

The arthrochalasia type of EDS (EDS type VIIA and B) is also caused by mutations in the gene encoding collagen I. It is characterized by severe generalized joint hypermobility with recurrent subluxations, skin hyperextensibility, osteopenia, and muscle hypotonia (*Table 2*).[53]

Defects of type III collagen cause the vascular type of EDS (EDS type IV), which is characterized by ruptures of arteries, hollow organs, tendons, and muscles, indicating a role of collagen III in muscle integrity.[53] Muscle involvement consisting of hypotonia, distal atrophy, muscle cramps and pain, and increased muscle ultrasound intensity have been described in a in a family with the vascular type EDS and a mosaic for the *COL3A1* mutation (*Table 2*).[57]

Collagen V and Ehlers-Danlos syndrome

Collagen V is a minor component of muscle ECM. Collagen V co-polymerizes with collagen type I and as such is predominantly located in the reticular lamina and beyond (*Figure 1*). Defects of collagen V cause the classical type of EDS (EDS I / II): mutations in the genes *COL5A1* and *COL5A2*, (coding for α1(V) and α2(V) collagen chains) disrupt the formation of heterotypic fibrils, resulting in abnormal diameter and packing of collagen fibrils.[53,58] Clinically, classical type EDS is characterized by skin hyperextensibility, widened atrophic scarring, both joint hypermobility (fingers, knees, elbows, trunk), and muscle hypotonia. Weak, hypotonic muscles with deep tendon hyporeflexia are commonly reported. Fatigue is another frequent complaint,[58] and severe muscle cramps, particularly at night have been described (*Table 2*). As with the other collagen disorders, muscular symptoms in the classical type EDS are generally explained as a result of increased extensibility of tendons and avoidance of exercise because of joint hypermobility. Furthermore, muscle fibres surrounded by defective collagen might lose their parallel orientation upon contraction, thus decreasing mechanical integrity and resulting in muscle weakness.[58] Finally, dysfunction of the Golgi tendon organ embedded in the loose tendons may play a role in determining muscle hypotonia, and thus contribute to fatigue and muscle weakness.[58]

Collagen IX and multiple epiphyseal dysplasia with mild myopathy

Type IX collagen is a heterotrimer that belongs to the fibril-associated collagens with interrupted triple helices. It is predominantly found in cartilage, where it is probably involved in the organization and spacing of type II collagen fibrils. It is a minor component of muscle ECM. Ultrastructurally, it is located in the reticular lamina and in the more peripheral ECM (*Figure 1*).

Mutations in the genes encoding two of the three helices result in multiple epiphyseal dysplasia (MED), a group of autosomal dominant skeletal dysplasias characterized by early-onset osteoarthritis, a waddling gait, and sometimes short stature (*Table 2*). MED with minimal hip involvement, predominant knee involvement, and evidence for a mild proximal myopathy was reported as a result of another mutation in *COL9A3*. Muscle biopsy revealed increased fibre diameter variance in both type I and II muscle fibres, representing myopathic changes.[59]

Tenascins and Ehlers-Danlos syndrome

Tenascins are very large glycoproteins with molecular weights ranging from 220 to 500 kDa. Vertebrates express four tenascins in their connective tissues (tenascin-C, -R, -X, and -W). So far, tenascin-C and –X were found to be expressed in skeletal muscle.[60] All tenascins have a similar structure, consisting of a cysteine-rich N-terminal domain, followed by a series of EGF-like repeats, multiple fibronectin type-III repeats, and a C-terminal globular domain homologous to fibrinogen. Their complex domain structure allows tenascins to interact with a variety of ECM proteins, notably with members of the integrin family.

Tenascin-C and mice model

In skeletal muscle, tenascin-C is found in the endomysium close to the myotendinous junction and in muscle spindles.[60] Tenascin-C was found to be upregulated in UCMD, suggesting that abnormal expression of proteoglycans and adhesion molecules may be involved in the pathogenesis of the myopathy in UCMD.[61] Tenascin-C deficient mice show reduced muscle strength.[62]

Tenascin-X and Ehlers-Danlos syndrome

TNX is present in the endo-, peri-, and epimysium. Its complex structure enables multiple interactions with other ECM (glyco)proteins, rendering TNX a crucial player in the organisation of the skeletal muscle ECM. Ultrastructurally, it is predominantly located in the reticular lamina and beyond (*Figure 1*).[63] Disruption of the *TNXB* gene significantly decreases the expression of type VI collagen. Moreover, together with other ECM molecules, TNX and type VI collagen are collectively involved in collagen fibrillogenesis *in vitro* and *in vivo*.[64]

Deficiency of TNX results in a phenotype similar to the classical type of EDS, but with a recessive inheritance and lack of skin hyperextensibility.[65,66] Clinical features resemble those seen in collagen VI myopathies.[10] Haploinsufficiency of the TNX gene causes hypermobility type EDS in only a minority of patients.[67] Muscle weakness and distal contractures as seen in

collagen VI myopathies have been reported in TNX-deficient EDS.[10,68] Quantitative muscle function in TNX-deficient EDS patients proved severely reduced despite normal findings on electromyography and muscle biopsy. Most likely, alterations in the ECM modify force transmission by the muscle connective tissue and thus influence muscle function in EDS.[68]

Fibrillin, fibulins, elastin, cutis laxa, and Marfan syndrome

Tissues that require elasticity in addition to tensile strength have a network of elastic fibres interwoven with the collagen network. The network of elastic fibres in the ECM of the fascia gives muscle the ability to recoil after transient stretch.[69] Elastin consists of many soluble tropoelastin protein molecules, which are linked to form an insoluble, cross-linked array of rubber-like fibres. Microfibrillar molecules (fibrillin-1, fibrillin-2, fibulin-1, fibulin-4, and fibulin-5) contribute to the pro-elastin fibre assembly, structure, and function.[70] They are also associated with collagen VI in tendons and are predominantly located in the reticular lamina and in the more peripheral ECM.

Structural or functional defects of the elastic fibre system may cause cutis laxa syndrome. Cutis laxa syndrome is a rare, genetically heterogeneous condition presenting in the early months of life with loose and redundant skin folds, decreased elasticity of the skin, and a variable spectrum of associated features, including generalized muscle weakness and hypotonia. Pathophysiological mechanisms in cutis laxa include fibulin-4 and -5 mutations, elastin mutations, lysyl oxidase deficiency, and abnormal glycosylation of fibulin or other N- and O-linked glycoproteins.[54,70]

Fibrillin-1 microfibrils are distributed throughout the muscle ECM and provide muscle with low-range elasticity (*Figure 1*). Mutations in the fibrillin-1 gene (*FBN1*) are associated with Marfan syndrome.[71] More than 1000 mutations have been identified and most of them are unique to an affected individual or family. Marfan considered muscle involvement integral to his syndrome and initial reports mentioned muscle hypoplasia. However, over the years myopathy disappeared from the nosology and was no longer included in the diagnostic criteria.[73] Only recently, muscle involvement in Marfan syndrome has received renewed attention. Behan *et al.* reported a family of which members confirmed to the diagnostic criteria, and had additional muscle weakness associated with respiratory failure. Muscle biopsy revealed defective fibrillin distribution in endomysium, with presence of truncated forms of fibrillin in restriction mapping.[8] Furthermore, the majority of individuals with Marfan syndrome lacks the ability to increase muscle mass in response to growth and exercise. Patients with early onset of severe and rapidly progressing Marfan syndrome have profound muscle hypoplasia and hypotonia throughout life (*Table 2*).[4,8,74] This is probably related to excess availability and signalling of active transforming growth factor β (TGF-β), which is normally kept inactive by fibrillin via TGF-β binding to the fibrillin associated latent TGF-β binding protein. Deficiency of fibrillin thus results in an excess activity of TGF-β, which may

impair satellite cell proliferation and differentiation. This probably contributes to the formation of fibrosis in response to injury, inflammation, or disease.[4] Recently, losartan, which antagonizes TGF- β, was found to prevent aortic aneurysm and to reverse muscle involvement in a mouse model of Marfan syndrome.[4]

Mutations in the fibrillin-2 gene (*FBN2*) cause the clinical syndrome of congenital contractural arachnodactyly (CCA), which shows phenotypic overlap with Marfan syndrome and is also characterized by muscle hypotonia, which might be neurogenic in origin.[75]

Perlecan, Schwartz-Jampel syndrome and Dyssegmental Dysplasia

Perlecan is a modular proteoglycan that is ubiquitously present in ECM and is one of the largest single-chain polypeptides known to date (470 kDa). The perlecan core protein is heavily glycosylated by numerous *O*-linked oligosaccharides and four heparan sulphate chains (fully glycosylated it can reach a molecular weight of over 800 kDa). Perlecan plays an important role at the neuromuscular junction, where it co-localizes with the acetylcholine receptors in newly forming synapses and anchors acetylcholine esterase through its collagen-Q tail.[76] Perlecan is involved in stabilization of molecules within the extrasynaptic ECM, in glomerular permeability, and in cell adhesion. Perlecan can directly interact with a wide variety of ECM molecules, including type IV collagen, fibronectin, and laminin (*Figure 1*).

Mutations in the human perlecan gene (*HSPG2*) result in a spectrum of perlecan-related disorders, with Schwartz-Jampel syndrome (SJS) at the moderate severe side and Silverman Handmaker syndrome (SHS) at the most extreme end.[77] These disorders are characterized by chondrodysplasia, bone dysplasia, congenital bowing of shortened femora and tibiae, and facial manifestations consisting of a small mouth, micrognathia, blepharophimosis, and pursed lips. A recent study in perlecan-null mice demonstrated that clustering molecules, such as acetylcholine receptor and agrin, were present at the neuromuscular junction but that acetylcholine esterase was completely absent, indicating that perlecan is a key molecule for localizing acetylcholine esterase at the synapse.[78] Reduced amounts of either truncated or normal perlecan molecules may result in reduced clustering of acetylcholine esterase at the neuromuscular junction in SJS, which would likely cause a greater concentration of acetylcholine. This would stimulate acetylcholine receptor activity and thus cause myotonia (*Table 2*).[78]

Biglycan, decorin and mice models

Biglycan and decorin are small proteoglycans with a highly homologous amino acid sequence, distinguished by the presence of one (decorin) or two (biglycan) chontroitin or dermatan sulphate side chains. Numerous functions have been documented including cell adhesion and signal transduction. Both biglycan and decorin have the ability to interact with collagens to assemble supramolecular structures (biglycan with type I and VI collagen; decorin with type I, II, III, and VI collagen).[79] Biglycan also binds to alpha- and gamma

sarcoglycan. Decorin mediates the association of TNX with collagen fibrils and is mainly found in the ECM, whereas biglycan is localized more closely associated with sarcoglycans near the sarcolemma.

Biglycan deficiency leads to structural abnormality in collagen fibrils in bone, dermis, and tendon. Biglycan null mice have a reduced bone mass and an osteoporosis-like phenotype with growth failure and thinning of the dermis but without overt skin fragility.[80] Decorin null mice also have structural abnormalities of collagen fibrils and present with lax skin of markedly reduced tensile strength with a thin dermis, similar to that observed in the human EDS.[80] Neither biglycan nor decorin gene mutations are known in human. However, their expression is notably altered in several muscular dystrophies. Variations in the transcript and protein levels of these proteoglycans in DMD and MDC1A probably reflect the disruption of ECM organization that occurs in these diseases, and the role of both molecules in the muscle response to this dystrophic cell damage.[79] Furthermore, biglycan has an important function during muscle and connective tissue development, and it may play a role in the pathogenesis of collagen VI-associated congenital muscular dystrophies.[81]

Summarizing remarks

In conclusion, we have summarized some of the present knowledge about ECM molecules present in muscle and of the diseases associated with defects in these molecules, which include both myopathies and ICTDs. These disorders lead to considerable muscle weakness in many cases and pose great differential diagnostic challenges. Future studies on patients and experimental animal models may further reveal the phenotypic spectrum and the pathogenic mechanism of these disorders.

Reference List

1. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. J Pathol 2003; 200: 423-428.
2. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 2002; 13: 377-383.
3. Carmeli E, Moas M, Reznick AZ, Coleman R. Matrix metalloproteinases and skeletal muscle: a brief review. Muscle Nerve 2004; 29: 191-197.
4. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007; 13: 204-210.
5. Schessl J, Zou Y, Bonnemann CG. Congenital muscular dystrophies and the extracellular matrix. Semin Pediatr Neurol 2006; 13: 80-89.
6. Jobsis GJ, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA. Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. Nat Genet 1996; 14: 113-115.
7. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, Anton-Lamprecht I, Muntoni F, Hanefeld F, Bonnemann CG. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A 2005; 132: 296-301.
8. Behan WM, Longman C, Petty RK, Comeglio P, Child AH, Boxer M, Foskett P, Harriman DG. Muscle fibrillin deficiency in Marfan's syndrome myopathy. J Neurol Neurosurg Psychiatry 2003; 74: 633-638.
9. Bondestam J, Pihko H, Vanhanen SL, Brander A, Toiviainen-Salo S, Marttinen E, Makitie O. Skeletal dysplasia presenting as a neuromuscular disorder - report of three children. Neuromuscul Disord 2007; 17: 231-234.
10. Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Ehlers-Danlos syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies. Am J Med Genet A 2007; 143: 2215-2219.
11. Zou Y, Zhang RZ, Sabatelli P, Chu ML, Bonnemann CG. Muscle interstitial fibroblasts are the main source of collagen VI synthesis in skeletal muscle: implications for congenital muscular dystrophy types Ullrich and Bethlem. J Neuropathol Exp Neurol 2008; 67: 144-154.
12. Nishimura T, Hattori A, Takahashi K. Ultrastructure of the intramuscular connective tissue in bovine skeletal muscle. A demonstration using the cell-maceration/scanning electron microscope method. Acta Anat (Basel) 1994; 151: 250-257.
13. Sanes JR. The extracellular matrix. In: Engel A.G., Franzini-Armstrong C., editors. Myology. Basic and Clinical. New York: Mc Graw Hill Medical Publishing Edition; 2004.
14. Huijing PA, Jaspers RT. Adaptation of muscle size and myofascial force transmission: a review and some new experimental results. Scand J Med Sci Sports 2005; 15: 349-380.
15. Kjaer M, Magnusson P, Krogsgaard M, Boysen MJ, Olesen J, Heinemeier K, Hansen M, Haraldsson B, Koskinen S, Esmarck B, Langberg H. Extracellular matrix adaptation of tendon and skeletal muscle to exercise. J Anat 2006; 208: 445-450.
16. Davies KE, Nowak KJ. Molecular mechanisms of muscular dystrophies: old and new players. Nat Rev Mol Cell Biol 2006; 7: 762-773.
17. Muntoni F, Voit T. The congenital muscular dystrophies in 2004: a century of exciting progress. Neuromuscul Disord 2004; 14: 635-649.
18. Campbell KP, Stull JT. Skeletal muscle basement membrane-sarcolemma-cytoskeleton interaction minireview series. J Biol Chem 2003; 278: 12599-12600.
19. Lampe AK, Bushby KM. Collagen VI related muscle disorders. J Med Genet 2005; 42: 673-685.
20. Laval SH, Bushby KM. Limb-girdle muscular dystrophies--from genetics to molecular pathology. Neuropathol Appl Neurobiol 2004; 30: 91-105.
21. Yoshida T, Pan Y, Hanada H, Iwata Y, Shigekawa M. Bidirectional signaling between sarcoglycans and the integrin adhesion system in cultured L6 myocytes. J Biol Chem 1998; 273: 1583-1590.
22. Ingber DE. Tensegrity II. How structural networks influence cellular information processing networks. J Cell Sci 2003; 116: 1397-1408.
23. Kanagawa M, Toda T. The genetic and molecular basis of muscular dystrophy: roles of cell-matrix linkage in the pathogenesis. J Hum Genet 2006; 51: 915-926.

24. Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, Dollar J, Nishino I, Kelley RI, Somer H, Straub V, Mathews KD, Moore SA, Campbell KP. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. Nature 2002; 418: 417-422.
25. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: an overview of current mouse models. Curr Opin Genet Dev 2002; 12: 349-361.
26. Herzog C, Has C, Franzke CW, Echtermeyer FG, Schlotzer-Schrehardt U, Kroger S, Gustafsson E, Fassler R, Bruckner-Tuderman L. Dystroglycan in skin and cutaneous cells: beta-subunit is shed from the cell surface. J Invest Dermatol 2004; 122: 1372-1380.
27. Brockington M, Blake DJ, Prandini P, Brown SC, Torelli S, Benson MA, Ponting CP, Estournet B, Romero NB, Mercuri E, Voit T, Sewry CA, Guicheney P, Muntoni F. Mutations in the fukutin-related protein gene (FKRP) cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan. Am J Hum Genet 2001; 69: 1198-1209.
28. Barresi R, Michele DE, Kanagawa M, Harper HA, Dovico SA, Satz JS, Moore SA, Zhang W, Schachter H, Dumanski JP, Cohn RD, Nishino I, Campbell KP. LARGE can functionally bypass alpha-dystroglycan glycosylation defects in distinct congenital muscular dystrophies. Nat Med 2004; 10: 696-703.
29. Allikian MJ, McNally EM. Processing and assembly of the dystrophin glycoprotein complex. Traffic 2007; 8: 177-183.
30. Ozawa E, Mizuno Y, Hagiwara Y, Sasaoka T, Yoshida M. Molecular and cell biology of the sarcoglycan complex. Muscle Nerve 2005; 32: 563-576.
31. Taniguchi M, Kurahashi H, Noguchi S, Sese J, Okinaga T, Tsukahara T, Guicheney P, Ozono K, Nishino I, Morishita S, Toda T. Expression profiling of muscles from Fukuyama-type congenital muscular dystrophy and laminin-alpha 2 deficient congenital muscular dystrophy; is congenital muscular dystrophy a primary fibrotic disease? Biochem Biophys Res Commun 2006; 342: 489-502.
32. Boppart MD, Burkin DJ, Kaufman SJ. Alpha7beta1-integrin regulates mechanotransduction and prevents skeletal muscle injury. Am J Physiol Cell Physiol 2006; 290: C1660-C1665.
33. Rooney JE, Welser JV, Dechert MA, Flintoff-Dye NL, Kaufman SJ, Burkin DJ. Severe muscular dystrophy in mice that lack dystrophin and alpha7 integrin. J Cell Sci 2006; 119: 2185-2195.
34. Mayer U. Integrins: redundant or important players in skeletal muscle? J Biol Chem 2003; 278: 14587-14590.
35. Chernousov MA, Kaufman SJ, Stahl RC, Rothblum K, Carey DJ. alpha7beta1 integrin is a receptor for laminin-2 on Schwann cells. Glia 2007; 55: 1134-1144.
36. Flintoff-Dye NL, Welser J, Rooney J, Scowen P, Tamowski S, Hatton W, Burkin DJ. Role for the alpha7beta1 integrin in vascular development and integrity. Dev Dyn 2005; 234: 11-21.
37. Mayer U, Saher G, Fassler R, Bornemann A, Echtermeyer F, von der Mark H, Miosge N, Poschl E, von der Mark K. Absence of integrin alpha 7 causes a novel form of muscular dystrophy. Nat Genet 1997; 17: 318-323.
38. Hayashi YK, Chou FL, Engvall E, Ogawa M, Matsuda C, Hirabayashi S, Yokochi K, Ziober BL, Kramer RH, Kaufman SJ, Ozawa E, Goto Y, Nonaka I, Tsukahara T, Wang JZ, Hoffman EP, Arahata K. Mutations in the integrin alpha7 gene cause congenital myopathy. Nat Genet 1998; 19: 94-97.
39. Miner JH, Yurchenco PD. Laminin functions in tissue morphogenesis. Annu Rev Cell Dev Biol 2004; 20: 255-284.
40. Hayashi YK, Tezak Z, Momoi T, Nonaka I, Garcia CA, Hoffman EP, Arahata K. Massive muscle cell degeneration in the early stage of merosin-deficient congenital muscular dystrophy. Neuromuscul Disord 2001; 11: 350-359.
41. Gawlik KI, Mayer U, Blomberg K, Sonnenberg A, Ekblom P, Durbeej M. Laminin alpha1 chain mediated reduction of laminin alpha2 chain deficient muscular dystrophy involves integrin alpha7beta1 and dystroglycan. FEBS Lett 2006; 580: 1759-1765.
42. Pan TC, Zhang RZ, Sudano DG, Marie SK, Bonnemann CG, Chu ML. New molecular mechanism for Ullrich congenital muscular dystrophy: a heterozygous in-frame deletion in the COL6A1 gene causes a severe phenotype. Am J Hum Genet 2003; 73: 355-369.
43. Bonaldo P, Braghetta P, Zanetti M, Piccolo S, Volpin D, Bressan GM. Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy. Hum Mol Genet 1998; 7: 2135-2140.
44. Baker NL, Morgelin M, Pace RA, Peat RA, Adams NE, Gardner RJ, Rowland LP, Miller G, De Jonghe P, Ceulemans B, Hannibal MC, Edwards M, Thompson EM, Jacobson R, Quinlivan RC, Aftimos S, Kornberg AJ, North KN, Bateman JF,

Lamande SR. Molecular consequences of dominant Bethlem myopathy collagen VI mutations. Ann Neurol 2007; 62: 390-405.
45. Camacho VO, Bertini E, Zhang RZ, Petrini S, Minosse C, Sabatelli P, Giusti B, Chu ML, Pepe G. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. Proc Natl Acad Sci U S A 2001; 98: 7516-7521.
46. Ullrich O. Kongenitale, atonisch-sklerotische Muskeldystrophie. 1930. Monatschr Kinderheilkd 1930; 47:502-10.
47. Kvist AP, Latvanlehto A, Sund M, Eklund L, Vaisanen T, Hagg P, Sormunen R, Komulainen J, Fassler R, Pihlajaniemi T. Lack of cytosolic and transmembrane domains of type XIII collagen results in progressive myopathy. Am J Pathol 2001; 159: 1581-1592.
48. Hagg P, Vaisanen T, Tuomisto A, Rehn M, Tu H, Huhtala P, Eskelinen S, Pihlajaniemi T. Type XIII collagen: a novel cell adhesion component present in a range of cell-matrix adhesions and in the intercalated discs between cardiac muscle cells. Matrix Biol 2001; 19: 727-742.
49. Myers JC, Dion AS, Abraham V, Amenta PS. Type XV collagen exhibits a widespread distribution in human tissues but a distinct localization in basement membrane zones. Cell Tissue Res 1996; 286: 493-505.
50. Halfter W, Dong S, Schurer B, Cole GJ. Collagen XVIII is a basement membrane heparan sulfate proteoglycan. J Biol Chem 1998; 273: 25404-25412.
51. Marneros AG, Olsen BR. Physiological role of collagen XVIII and endostatin. FASEB J 2005; 19: 716-728.
52. Eklund L, Piuhola J, Komulainen J, Sormunen R, Ongvarrasopone C, Fassler R, Muona A, Ilves M, Ruskoaho H, Takala TE, Pihlajaniemi T. Lack of type XV collagen causes a skeletal myopathy and cardiovascular defects in mice. Proc Natl Acad Sci U S A 2001; 98: 1194-1199.
53. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
54. Morava E, Wopereis S, Coucke P, Gillessen-Kaesbach G, Voit T, Smeitink J, Wevers R, Grunewald S. Defective protein glycosylation in patients with cutis laxa syndrome. Eur J Hum Genet 2005; 13: 414-421.
55. Rauch F, Glorieux FH. Osteogenesis imperfecta. Lancet 2004; 363: 1377-1385.
56. Engelbert RH, Uiterwaal CS, Gerver WJ, van der Net JJ, Pruijs HE, Helders PJ. Osteogenesis imperfecta in childhood: impairment and disability. A prospective study with 4-year follow-up. Arch Phys Med Rehabil 2004; 85: 772-778.
57. Palmeri S, Mari F, Meloni I, Malandrini A, Ariani F, Villanova M, Pompilio A, Schwarze U, Byers PH, Renieri A. Neurological presentation of Ehlers-Danlos syndrome type IV in a family with parental mosaicism. Clin Genet 2003; 63: 510-515.
58. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
59. Bonnemann CG, Cox GF, Shapiro F, Wu JJ, Feener CA, Thompson TG, Anthony DC, Eyre DR, Darras BT, Kunkel LM. A mutation in the alpha 3 chain of type IX collagen causes autosomal dominant multiple epiphyseal dysplasia with mild myopathy. Proc Natl Acad Sci U S A 2000; 97: 1212-1217.
60. Chiquet-Ehrismann R, Tucker RP. Connective tissues: signalling by tenascins. Int J Biochem Cell Biol 2004; 36: 1085-1089.
61. Higashi K, Higuchi I, Niiyama T, Uchida Y, Shiraishi T, Hashiguchi A, Saito A, Horikiri T, Suehara M, Arimura K, Osame M. Abnormal expression of proteoglycans in Ullrich's disease with collagen VI deficiency. Muscle Nerve 2006; 33: 120-126.
62. Morellini F, Schachner M. Enhanced novelty-induced activity, reduced anxiety, delayed resynchronization to daylight reversal and weaker muscle strength in tenascin-C-deficient mice. Eur J Neurosci 2006; 23: 1255-1268.
63. Lethias C, Carisey A, Comte J, Cluzel C, Exposito JY. A model of tenascin-X integration within the collagenous network. FEBS Lett 2006; 580: 6281-6285.
64. Minamitani T, Ikuta T, Saito Y, Takebe G, Sato M, Sawa H, Nishimura T, Nakamura F, Takahashi K, Ariga H, Matsumoto K. Modulation of collagen fibrillogenesis by tenascin-X and type VI collagen. Exp Cell Res 2004; 298: 305-315.
65. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.

66. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
67. Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M, Kucharekova M, Boezeman JB, Schalkwijk J. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet 2003; 73: 214-217.
68. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
69. Chu ML, Tsuda T. Fibulins in development and heritable disease. Birth Defects Res C Embryo Today 2004; 72: 25-36.
70. Hucthagowder V, Sausgruber N, Kim KH, Angle B, Marmorstein LY, Urban Z. Fibulin-4: a novel gene for an autosomal recessive cutis laxa syndrome. Am J Hum Genet 2006; 78: 1075-1080.
71. Dietz HC, Pyeritz RE. Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome and related disorders. Hum Mol Genet 1995; 4 Spec No: 1799-1809.
72. Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, Gautier E, Callewaert B, Arbustini E, Mayer K, rslan-Kirchner M, Kiotsekoglou A, Comeglio P, Marziliano N, Dietz HC, Halliday D, Beroud C, Bonithon-Kopp C, Claustres M, Muti C, Plauchu H, Robinson PN, Ades LC, Biggin A, Benetts B, Brett M, Holman KJ, De Backer J, Coucke P, Francke U, De Paepe A, Jondeau G, Boileau C. Effect of mutation type and location on clinical outcome in 1,013 probands with Marfan syndrome or related phenotypes and FBN1 mutations: an international study. Am J Hum Genet 2007; 81: 454-466.
73. De Paepe AM, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996; 62: 417-426.
74. Percheron G, Fayet G, Ningler T, Le Parc JM, Denot-Ledunois S, Leroy M, Raffestin B, Jondeau G. Muscle strength and body composition in adult women with Marfan syndrome. Rheumatology (Oxford) 2007; 46: 957-962.
75. Tuncbilek E, Alanay Y. Congenital contractural arachnodactyly (Beals syndrome). Orphanet J Rare Dis 2006; 1: 20.
76. Peng HB, Xie H, Rossi SG, Rotundo RL. Acetylcholinesterase clustering at the neuromuscular junction involves perlecan and dystroglycan. J Cell Biol 1999; 145: 911-921.
77. Nicole S, Davoine CS, Topaloglu H, Cattolico L, Barral D, Beighton P, Hamida CB, Hammouda H, Cruaud C, White PS, Samson D, Urtizberea JA, Lehmann-Horn F, Weissenbach J, Hentati F, Fontaine B. Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia). Nat Genet 2000; 26: 480-483.
78. Arikawa-Hirasawa E, Rossi SG, Rotundo RL, Yamada Y. Absence of acetylcholinesterase at the neuromuscular junctions of perlecan-null mice. Nat Neurosci 2002; 5: 119-123.
79. Zanotti S, Negri T, Cappelletti C, Bernasconi P, Canioni E, Di Blasi C, Pegoraro E, Angelini C, Ciscato P, Prelle A, Mantegazza R, Morandi L, Mora M. Decorin and biglycan expression is differentially altered in several muscular dystrophies. Brain 2005; 128: 2546-2555.
80. Corsi A, Xu T, Chen XD, Boyde A, Liang J, Mankani M, Sommer B, Iozzo RV, Eichstetter I, Robey PG, Bianco P, Young MF. Phenotypic effects of biglycan deficiency are linked to collagen fibril abnormalities, are synergized by decorin deficiency, and mimic Ehlers-Danlos-like changes in bone and other connective tissues. J Bone Miner Res 2002; 17: 1180-1189.
81. Lechner BE, Lim JH, Mercado ML, Fallon JR. Developmental regulation of biglycan expression in muscle and tendon. Muscle Nerve 2006; 34: 347-355.

PART II

Neuromuscular features of Ehlers-Danlos syndrome

A

Clinical evaluation of Ehlers-Danlos syndrome patients

CHAPTER 3

Initial clinical observations in Ehlers-Danlos syndrome

Report 1

Recurrent neuropathy associated with Ehlers-Danlos syndrome

Report 2

Myopathy and polyneuropathy in an adolescent with the kyphoscoliotic type of Ehlers-Danlos syndrome

Report 3

Ehlers-Danlos Syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies

CHAPTER 3

Report 1: Recurrent neuropathy associated with Ehlers-Danlos syndrome

Adapted from:
Voermans NC, Drost G, van Kampen A, Gabreëls-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG.
J Neurol 2006;253:670-1.

Abstract

Peripheral nervous system involvement in Ehlers-Danlos syndrome has been reported only sporadically. We describe a patient with the hypermobility type of Ehlers-Danlos syndrome who sequentially suffered from an axillary neuropathy, a brachial plexopathy, and a sciatic neuropathy. We shortly discuss the possible pathophysiological mechanisms involved.

Introduction

The Ehlers-Danlos syndrome (EDS) is a group of inherited connective tissue disorders (ICTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility resulting in recurrent joint dislocations, vascular lesions, easy bruising, and excessive scarring.[1] The hypermobility type of EDS is restricted to mild skin involvement and generalized joint hypermobility. EDS is associated with deficiency of extracellular matrix (ECM) proteins such as collagen I, III and V and tenascin-X (TNX), but in the hypermobility type the protein deficiency is mostly unknown.[1-3] Although initially described by Beighton in 1970,[4] peripheral nervous system involvement in EDS has only been reported sporadically.[5-7] We here report a patient with the hypermobility type of EDS who subsequently experienced an axillary neuropathy, a brachial plexopathy, and a sciatic neuropathy.

Case report

A 30-year-old female with the hypermobility type of EDS (former type III) presented with acute onset of paresis and sensory disturbances of her lower left leg. The previous evening, she had fallen asleep while sitting cross-legged. Sedation, which occurred as a side effect of analgesics probably enabled her to fall asleep in this seemingly awkward position. When she woke up after approximately four hours, she experienced weakness and numbness of her lower left leg. This had not recovered the other day when she was admitted to the emergency room.

EDS was diagnosed at the age of 20. She had suffered from recurrent dislocations of her right shoulder, which were initially complicated by an axillary neuropathy. Gradually, the dislocation became irreversible and was accompanied by increasing numbness and paresthesias in her whole arm probably due to stretching of the brachial plexus. Both the axillary neuropathy and brachial plexopathy were confirmed by nerve conduction studies and electromyography. She underwent a series of fixative operations of her right shoulder. Nevertheless, due to recurrent dislocations, post-operative infections, and a continual brachial plexopathy she decided to have her right arm amputated at the age of 27. Since then, she preferred sitting cross-legged to increase her balance. Neuropathological studies of the ulnar and radial nerve had then revealed no signs of infection nor myxoid degeneration. The perineurium and endoneurium appeared normal. There was no more ultrastructural material available so that a still more refined investigation could not be performed. Her family history was negative for EDS or Hereditary Neuropathy with Liability to Pressure Palsies. DNA analysis for 17p11.2 deletion was negative.

Physical examination upon arrival at the emergency room revealed joint hypermobility (Beighton score 7/9) and a smooth and velvety skin without striae or hyperextensibility. Knee flexion (Medical Research Council (MRC) 4)[8] and foot extension (MRC 2) were weak. Foot flexion and toe movements were absent. Sensory loss was present over the lateral lower leg and foot. Knee jerks were symmetrical; the left ankle jerk was absent.

Two sequential EMG examinations, performed on day 2 and 3 weeks later showed signs of a previous left peroneal neuropathy, probably due to her long-standing habit of sitting cross-legged, and several findings that indicated a recent sciatic neuropathy in the same limb. These were: first absent and later delayed H-reflex latencies over the tibial nerve, normal findings in the gluteus medius muscle, and spontaneous activity in the tibialis anterior muscle after two weeks. She was discharged to a rehabilitation clinic for physical therapy and was given a foot-ankle-orthesis. Muscle strength improved gradually.

Discussion

The pathophysiological mechanism of peripheral neuropathy in the hypermobility type of EDS appears evident; i.e. hypermobility of joints causes abnormal stretching of or pressure on peripheral nerves, resulting in reversible neuropathy or plexopathy.[4,9] In addition, we suggested that increased vulnerability of peripheral nerves themselves to stretching or pressure due to the genetic connective tissue defect in EDS might also be involved. ECM proteins which are known to be involved in EDS, such as collagen I, III, V and TNX, are distributed throughout the connective tissue of peripheral nerves.[6,10,11] TNX or collagen deficient perineurium and endoneurium might fail to limit excessive stretching of or pressure on nerves. This might cause secondary axonal or myelin damage and impaired peripheral nerve function. Clinicians and patients need to be aware of this possible risk of neuropathies in EDS, especially in the hypermobility type of EDS, in order to prevent recurrent nerve injuries.

CHAPTER 3

Report 2: Myopathy and polyneuropathy in an adolescent with the kyphoscoliotic type of Ehlers-Danlos syndrome

Adapted from:
Voermans NC, Bönnemann CG, Lammens M, van Engelen BG, Hamel BC.
Am J Med Genet A 2009;149A:2311-6.

Abstract

Ehlers-Danlos syndrome (EDS) is the most prevalent inherited connective tissue disorder. It is genetically and clinically heterogeneous and six major types are identified. The kyphoscoliotic type of EDS is characterized by generalized joint hypermobility, severe muscle hypotonia at birth, progressive scoliosis, and ocular fragility.

We report a 16-year-old patient with the kyphoscoliotic type of EDS with generalized muscle weakness and hypotonia. Electromyography, muscle ultrasound, MRI, and muscle biopsy showed myopathic changes, and nerve conduction studies revealed a mild axonal polyneuropathy. Taken together, these findings point to considerable neuromuscular involvement in the kyphoscoliotic type of EDS beyond the neonatal period.

Introduction

Ehlers-Danlos syndrome (EDS) is one of the most prevalent ICTDs. The syndrome is divided into six major types, all of which are characterized by symptoms due to connective tissue weakness. Skin, joints and ligaments, blood vessels, and eyes are predominantly affected.[1,9] Former EDS type VI, now called the kyphoscoliotic type of EDS, is a rare, autosomal-recessive form of the disease presenting with progressive kyphoscoliosis already present at birth, ocular manifestations, and neonatal muscle hypotonia (*Table 1*). Therefore, this type of EDS should be considered in the differential diagnosis of the floppy infant.[9,12-14]

In the majority of cases, the symptoms have been biochemically attributed to a deficiency of lysyl hydroxylase 1 (*LH1*), a collagen modifying enzyme. This deficiency results in underhydroxylation of collagen lysyl residues; hence, an abnormal pattern of lysyl pyridinolines and hydroxylysyl pyridinoline crosslinks is excreted in the urine.[1] Homozygosity or compound heterozygosity for mutant *PLOD* allele(s) results in LH1 deficiency, and mutation analysis should be performed for molecular confirmation and to allow for prenatal diagnosis in subsequent pregnancies.[15]

We here report a patient with the kyphoscoliotic type of EDS, in whom in the neonatal period a neuromuscular disorder was suspected. Electromyography and muscle biopsy then revealed no abnormalities. At the age of 12, the kyphoscoliotic type of EDS was diagnosed. At the age of 16, generalized muscle weakness and hypotonia were present, and investigations revealed typical findings of myopathy and mild axonal polyneuropathy, both of which may have contributed to muscle weakness.

Case report

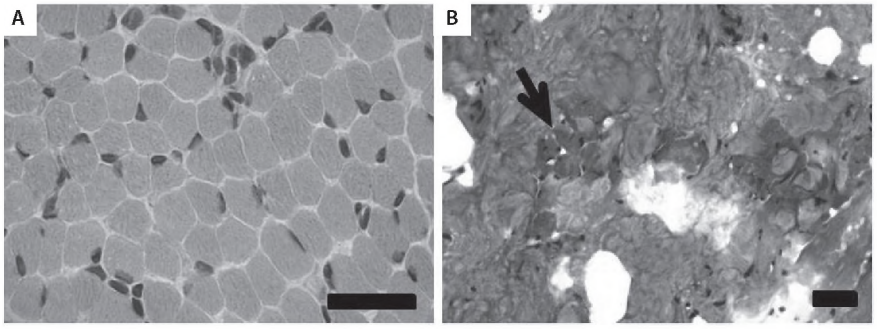
The patient is the first child of first cousins from the Netherlands. A neuromuscular disorder was considered initially because of the severe muscle hypotonia noted after birth; however, the neuromuscular work-up at that time, including muscle biopsy and electromyography, was normal (*Figure 1A*). Nevo syndrome was diagnosed at the age of 3 and the patient was reported by Hilderink and Brunner in 1995.[16] In 2005, Giunta et al. reported this case again and concluded that Nevo syndrome is allelic to and clinically indistinguishable from the kyphoscoliotic type of EDS (patient 7).[17] Mutation analysis of *PLOD1* showed a large homozygous deletion of exon 17, for which both parents were heterozygous. Neuromuscular findings noted in the perinatal period, infancy, and childhood are summarized in *Table 2* and illustrated in *Figure 1A*; for an extensive description we refer to the previous reports on this patient.[16,17]

Table 1 Diagnostic criteria of the kyphoscoliotic type EDS.[1] The presence of three major criteria in an infant is suggestive of the diagnosis, and laboratory and genetic testing is warranted.

Major criteria	Minor criteria	Comments
Generalized joint hypermobility Severe muscle hypotonia at birth Scoliosis at birth (progressive) Scleral fragility and rupture of the ocular globe	Tissue fragility, including atrophic scars Easy bruising Arterial rupture Marfanoid habitus Microcornea Radiologically considerable osteopenia Family history, i.e., affected sibs	Muscular hypotonia can be very pronounced and leads to delayed gross motor development The phenotype is most often severe, frequently resulting in loss of ambulation in the 2nd / 3rd decade There have been reports of a less severe form of the condition, with normal activity of LH1 and normal hydroxylysine content in the dermis

Figure 1 Histological images of the muscle biopsy of the vastus lateralis muscle.

A: Rectus femoris open muscle biopsy at age 2 months revealed no abnormalities. **B**: Rectus femoris needle muscle biopsy at age 16 years revealed fibrous and fatty tissue with very few remaining muscle fibres (arrow). Hematoxylin-Phloxin, Bar = 50 micrometer.

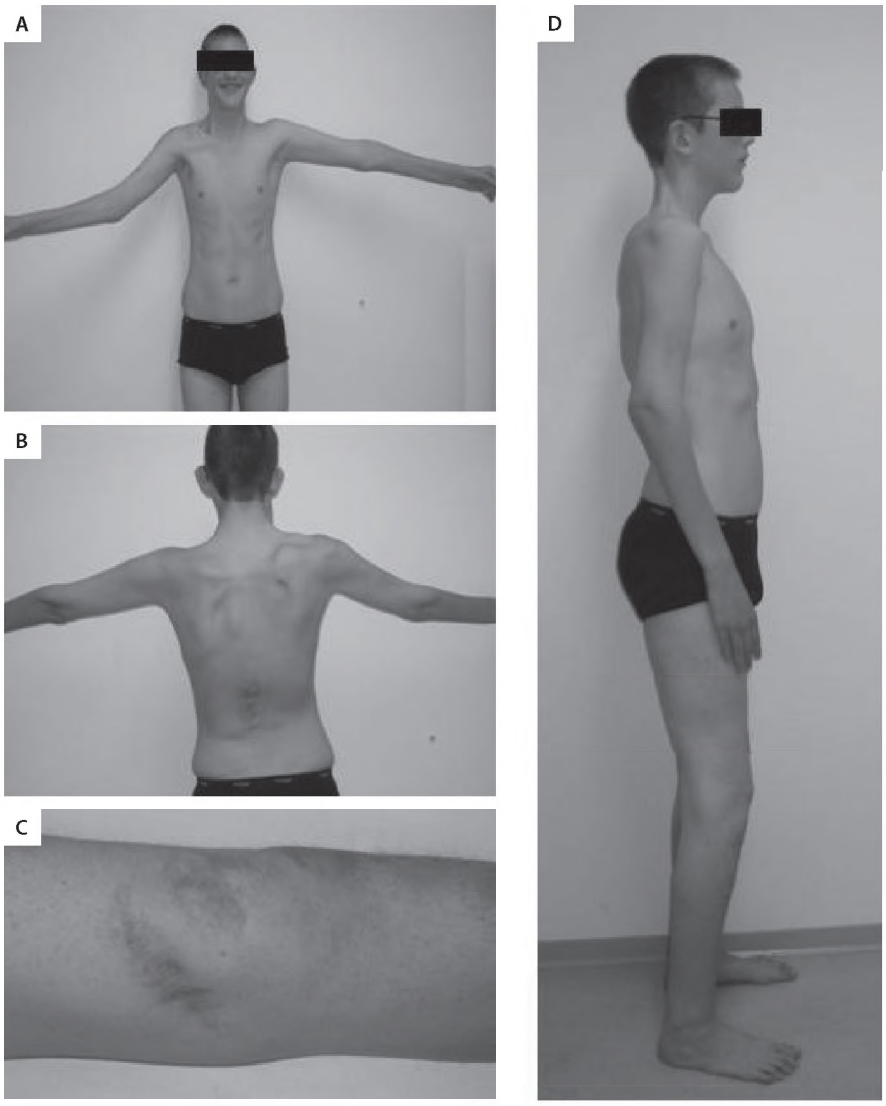


During puberty, the patient noticed that muscle strength and exercise tolerance were less than that of his peers, due to which he had difficulties walking, running, and cycling. He frequently noted myalgia after mild exercise; and walking distances was limited (< 500 m) due to easy fatigability. At the age of 15, he had a rupture of an aneurysm of the left popliteal artery, after which a stent was placed. Neurological examination (at the age of 16) revealed

generalized muscle weakness. The pattern of manual muscle testing scores (MRC scores) was confirmed by results of hand-held dynamometry (data not shown). Muscle mass was reduced and mild muscle hypotonia was still present (*Figure 2*). Deep tendon reflexes were

Figure 2 Clinical features at the age of 16.

A, **B**, and **C:** Maximal shoulder abduction with mild scapular winging on right side. Elbow contracture on right side. **D**: Atrophic scarring on knee.



symmetrically depressed. Vibration sense was reduced in hands and feet bilaterally.[18] Position sense of fingers and toes was normal. Coordination tests of arms were normal; tandem gait was mildly impaired. Furthermore, he had a hyperextensible skin with atrophic scars, a contracture of right elbow; and hypermobility of distal joints with a Beighton hypermobility score of 4/9 (a score of ≥ 5/9 indicates joint hypermobility)(*Table 2*).[1]

Creatine kinase (CK) was mildly increased (344 U/l; N < 220 U/l). Nerve conduction studies revealed low sensory nerve action potential amplitudes and compound muscle action potential amplitudes, with mildly reduced conduction velocities in arms and legs, compatible with a mild sensomotor axonal polyneuropathy with secondary slowing of conduction velocities (*Table 2*). Electromyography revealed small, polyphasic units in the biceps brachii, deltoid, and gluteus maximus muscles, reflecting myopathy. Laboratory investigations revealed no metabolic or toxic cause of polyneuropathy, and serum creatine kinase was mildly elevated (344 U/l; normal < 220 U/l). Muscle ultrasound revealed considerably increased echo intensity of the flexor muscles of the forearm, of the quadriceps muscle, and of the anterior tibial muscle bilaterally. Atrophy on muscle ultrasound was noted in most muscles examined.[19] T1-weighted muscle MRI revealed myopathic changes (increase of fat tissue and atrophy) of the erector spinae, gluteal, hamstrings, and calve muscles (*Figure 3*). Re-examination of the muscle biopsy performed at the age of 2 months of age revealed no abnormalities (*Figure 1A*). A second needle biopsy of the right quadriceps muscle at the age of 16 revealed fibrous and fatty tissue with very few atrophic muscle fibres (*Figure 1B*).

Figure 3 T1-weighted muscle muscle MRI.

A and **B:** Myopathic changes (increase of fat tissue and atrophy) of the erector spinae. **C:** Myopathic changes of the gluteal muscles. **D:** Myopathic changes of the hamstrings (unilateral arrows indicate myopathic changes).

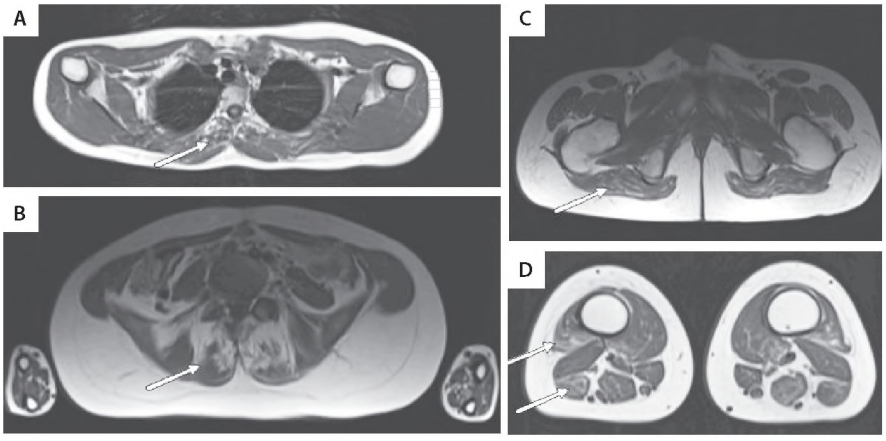


Table 2 Findings of physical examination and ancillary investigations.[16,17]

	Perinatal / Neonatal period	Infancy	Childhood
Physical examination	Reduced fetal movements Weak crying and sucking Severe generalized hypotonia with facial weakness Severe thoracolumbar kyphoscoliosis	Delayed motor development (rolling over at 13 months; walking unsupported at 22 months) Gradual improvement of hypotonia Mild facial weakness Muscle atrophy, distally most pronounced	Participates in all normal school activities except sports No progression of kyphoscoliosis
(Differential) diagnosis	Neuromuscular disorder Marfan syndrome	Nevo syndrome (age 3)	Kyphoscoliotic type EDS (age 12)

Adolescence							
Physical examination	*NCS*	*EMG*	*Muscle ultrasound*	*EI (Z)*	*MD (Z)*	*Muscle MRI (T1)*	*Needle biopsy*
Generalized muscle weakness (shoulder abduction and elbow flexion MRC 3; other proximal and distal limb muscles and trunk muscles MRC 4) Reduced muscle mass and muscle hypotonia Symmetrically low deep tendon reflexes Reduced vibration sense in hand and feet bilaterally (measured with Rydel Seiffer tuning fork: 3 and 2 respectively; normal values ≥ 6.5 and ≥ 4.5 respectively, on a scale of 0 (absent) to 8 (maximal)[18]	Mild axonal sensomotor polyneuropathy with mild secondary reduction of conduction velocities: Reduction of compound muscle action potential amplitude and velocity: - Motor: peroneal nerve (2.4 mV, 32.6 m/s; p5 of normal values 11.2 mV; 42.1 m/s) - Sensory: sural nerve (1.6 mV; 46.2 m/s; p5 of normal values 6.6 mV)	Myopathy: Motor unit action potentials: - Small units (3-8 ms; biceps brachii; gluteal and deltoid muscle), with polyphasia in biceps brachii muscle Turns and amplitudes analysis: myopathic in biceps brachii	Biceps brachii Flexors in forearm Quadriceps Anterior tibial muscle	0.2 4.6 5.5 2.6	- 5.0 - 3.9 - 7.1 2.6	Fatty infiltration of erector spinae muscle (abdominal level), gluteal maximus, hamstrings, gastrocnemius, and extensors in lower leg	Fibrous and fatty tissue with very few atrophic muscle fibres

EI: Echo intensity, expressed in Z-value; MD: muscle diameter, expressed in Z-value.[19,20]

Discussion

In short, the case presented here shows that considerable neuromuscular involvement may be present in the kyphoscoliotic type EDS in adolescence. Remarkably, a neuromuscular disorder was considered in the neonatal period in this patient, but was then excluded by a muscle biopsy and electromyography. The kyphoscoliotic type of EDS was diagnosed in childhood. In adolescence, generalized muscle weakness and reduced vibration sense were present, and muscle ultrasound, muscle MRI, and electromyography indicated considerable muscle involvement. Needle biopsy of the right quadriceps muscle revealed almost only fibrous and fatty tissue with very few atrophic muscle fibres, which corresponds with the abnormalities found in the muscle ultrasound. Laboratory investigations revealed mild increase of CK, indicating limited muscle fibre necrosis. Furthermore, nerve conduction studies indicated a mild axonal polyneuropathy with mild secondary slowing of conduction velocities. Both myopathy and polyneuropathy may contribute to muscle weakness in this patient.

In contrast to these findings, muscle weakness is not included in the diagnostic criteria of the kyphoscoliotic type of EDS (*Table 1*).[9,12,13] In addition, muscle weakness or polyneuropathy in this type of EDS has only rarely been reported;[21,22] Farag reported two sibling with the kyphoscoliotic type EDS with polyneuropathy and secondary muscle atrophy, without further specification of its cause.[22] Interesting though, loss of ambulation in 2nd or 3rd decade is added as a comment to the diagnostic criteria of this type, but without note or comment on whether muscle or peripheral nerve dysfunction may contribute to this. This functional decline in adolescence corresponds to the progression of symptoms in the patient we described. It may suggest that neuromuscular features in the kyphoscoliotic type of EDS progresses with aging. Furthermore, muscle weakness or easy fatigability is likely to remain unnoticed in patients who avoid intense exercise to prevent dermal, ocular or arterial damage, and who are generally not referred to a neuro(myo)logist after the neonatal period.

Presence of myopathy and polyneuropathy in this patient could have a different genetic cause since the consanguineous parents might carry another genetic defect affecting muscle and peripheral nerve structure and function. However, the absence of myopathic changes in the neonatal muscle biopsy makes the presence of another myopathy less likely. Alternatively, myopathy and polyneuropathy might also be associated with the role of LH1 in the ECM of muscle and peripheral nerve. LH1 catalyzes the hydroxylation of lysine residues, mainly but not exclusively in [X-Lys-Gly] triplets in collagen and proteins with collagen-like sequences. The hydroxylysine residues formed have two important functions: first, they are essential for the stability of the intermolecular cross links that provide the collagen fibrils with their tensile strength and mechanical stability.[23] Second, they serve as attachment sites for carbohydrate units, which have an important role in the regulation of collagen fibril formation

and morphology and in the assembly of type IV collagen networks. This collagen crosslink formation occurs in the ECM.[23] Deficiency of LH1 thus results in abnormal collagen morphology and cross linking, which may influence muscle function. In analogy, abnormal composition of endo-, peri- and epineurium may influence peripheral nerve function in this type of EDS, resulting in (poly)neuropathy.[24] This case suggests that both muscle and peripheral nerve dysfunction may increase with aging. However, the exact mechanism in which this occurs is still unclear.

Our findings are supported by findings in LH1 knockout mice (*Plod1*$^{-/-}$).[23] These animals show considerable generalized muscle weakness and hypotonia after the neonatal period, causing gait abnormalities, and reduction of spontaneous movements. These abnormalities are progressive with age and are explained as a combined result of laxity or dislocation of joints and muscle weakness. The muscle biopsy presented in this article reveals no signs of myopathy; however, only a longitudinal section is shown, which may fail to reveal mild myopathic features.[23]

In summary, we have presented a 16-year-old patient with the kyphoscoliotic type of EDS with mild generalized muscle weakness and reduced vibration sense. Ancillary investigations revealed typical signs of myopathy and mild axonal polyneuropathy. Remarkably, a neuromuscular disorder had been considered in the neonatal period due to hypotonia; however, electromyography and muscle biopsy at that time revealed no signs of myopathy. This case report may thus contribute to improved recognition of neuromuscular features in the kyphoscoliotic type of EDS beyond the neonatal period. Furthermore, it stresses the importance of research on the role of the extracellular in muscle and peripheral nerve function.[25]

CHAPTER 3

Report 3: Ehlers-Danlos syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies

Adapted from:
Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Am J Med Genet A 2007;143A:2215-9.

Abstract

Kirschner et al. reported 5 patients with Ullrich congenital muscular dystrophy and clinical characteristics typical of disorders of connective tissues such as Ehlers-Danlos syndrome (EDS). Electron microscopy of skin biopsies revealed alterations in collagen fibril morphology and an increase in extracellular material, similar to findings in skin biopsies of patients with EDS. The authors suggested that there is considerable clinical as well as morphological overlap between Ullrich congenital muscular dystrophy and inherited connective tissue disorders such as EDS.

We here report an EDS patient with a reverse presentation. She was previously diagnosed with tenascin-X (TNX) deficiency type of EDS and was referred to the neurologist for muscle weakness. Physical examination revealed both joint hypermobility, skin hyperextensibility, generalized muscle weakness, and distal contractures. Ancillary investigations showed normal CK and myopathic features on electromyography but not in the muscle biopsy. Qualitative evaluation of collagen VI staining of the muscle biopsy showed mildly reduced endomysial staining.

The combination of joint hypermobility, generalized muscle weakness, and contractures is also characteristic of collagen VI myopathies. Recent studies support the functional links between collagen VI and TNX. This case provides additional support for the clinical and biochemical overlap between collagen VI-related myopathies and the TNX-deficient type of EDS.

Introduction

In their article "Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes" Kirschner *et al.*[26] reported 5 patients with Ullrich congenital muscular dystrophy (UCMD) and clinical characteristics typical of ICTDs such as Ehlers-Danlos syndrome (EDS). Electron microscopy of skin biopsies revealed alterations in collagen fibril morphology and an increase in extracellular material, similar to findings in skin biopsies of patients with EDS. The authors suggested that there is considerable clinical as well as morphological overlap between UCMD and ICTDs such as EDS.

Here, we confirm the connection between myopathies and ICTDs from the opposite perspective. Recently, we had the opportunity to investigate a patient with autosomal recessive EDS due to TNX deficiency with a reverse presentation. She has previously been described as *Patient 1* by Schalkwijk in 2001, and recently as *Patient 1* by Voermans in 2007.[2,27]

This patient had been diagnosed with classical type of EDS at the age of 27 and TNX deficiency was diagnosed at the age of 46. She presented the clinical features of EDS as well as generalized muscle weakness and distal contractures. The combination of generalized muscle weakness and joint hypermobility is seen in UCMD, while distal contractures typically occur in Bethlem Myopathy. Both UCMD and Bethlem myopathy result from defects in collagen VI, an ECM component that depends on TNX for proper functioning. We describe the clinical phenotype and the results of ancillary investigations. Furthermore, we discuss some the results of recent studies that support the functional links between collagen VI and TNX. We suggest that this case provides additional support for the clinical and biochemical overlap between collagen VI-related myopathies and EDS related to TNX deficiency.

Case report

A now 50-year-old woman has suffered from mild joint hypermobility, skin hyperextensibility, and easy bruising since childhood. She experienced recurrent dislocations of her right shoulder and had developed haematomas on the soles of her feet after high jumping. She had never been good at running. Classical type of EDS (type I/II according to the classification at that time) was diagnosed at the age of 27. The diagnosis was specified as TNX deficiency type of EDS at the age of 46. Indeed, she had not presented atrophic scarring as is common in the classical type of EDS. Absence of TNX protein in serum was confirmed by ELISA. Mutation analysis revealed a homozygous 2-bp deletion in exon 8, which encodes the fourth fibronectin type-III repeat of the TNX protein. At that time, she was referred to the neurologist with weakness of her right hand and mild generalized weakness of arms and legs. Although mild muscle weakness of her arms had already been reported when she was originally

diagnosed with EDS at the age of 27, muscle weakness had become symptomatic around the age of 40 and has gradually increased since then. She was unable to walk up stairs, her walking endurance was limited to one hour, and she suffered from reduced gripping force, predominantly of the pincer grip.

A general physical examination at the age of 46 revealed generalized joint hypermobility (Beighton score 5/9), bruises of her fingers, bilateral pes planus, molluscoid pseudotumors on the lateral side of her feet, and hyperextensibility of her skin, which was smooth and velvety (*Figure 4*). She did not have atrophic scars. The neurological examination indicated generalized muscle hypotonia and generalized weakness of arm, leg, and neck flexor muscles (MRC 4). Muscle atrophy and weakness were most pronounced in her hands and lower arms (MRC 3), more on the right than on the left side. She had very mild flexion contractures of distal phalanges predominantly of the right hand. Tendon reflexes were symmetrically low.

CK was normal (88 U/l). Nerve conduction studies revealed signs of a mild left carpal tunnel syndrome, but were otherwise normal. Electromyography showed only mild non-specific changes without clear signs of either a myopathy or a neurogenic disorder. Needle biopsy of the quadriceps muscle showed neither significant myopathic changes nor signs of disuse. Guinea pig anti-human TNX antibodies were used to stain TNX within muscle ECM. Visual inspection revealed absence of TNX within muscle ECM.[28] Mice anti-human collagen VI antibodies (Chemicon Millipore, Billerica, USA) were used to stain collagen VI within muscle ECM. Visual inspection revealed decreased endomysial collagen VI staining (*Figure 5*).

This patient was investigated again at the age of 50. The severity of the contractures of her fingers had gradually increased (*Figure 6*), for which she used an orthosis intermittently. Electromyography was repeated and revealed apparent myopathic changes in the arm and hand muscles on both sides.

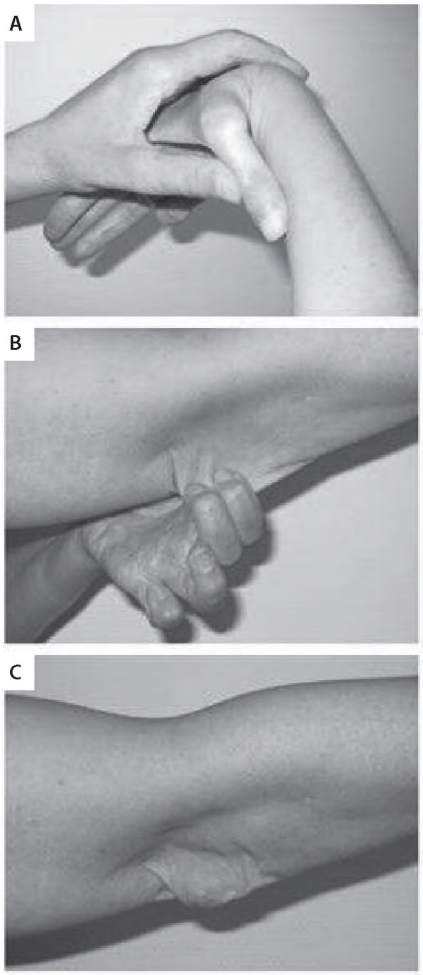
Discussion

In short, this patient shows clinical features that are characteristic of the TNX-deficiency type of EDS including joint hypermobility, skin hyperextensibility, and easy bruising.[2] The diagnosis was confirmed by the absence of TNX in serum and by mutation analysis. Starting at the age of 40, she gradually developed additional clinical symptoms that are frequently seen in collagen VI-related myopathies, including generalized muscle weakness and contractures of the distal phalanges of the hand.

TNX is an ECM-resident glycoprotein with a multidomain structure that consists of an N-terminal region involved in oligomerization, a series of epidermal growth factor-like repeats, a number of fibronectin-type III modules, and a C-terminal domain that is homologous

Figure 4 Typical characteristics of TNX-deficient EDS. **A:** Joint hypermobility. **B** and **C:** Skin hyperextensibility (this should preferably tested at the volar side of the forearm).

Figure 5 Muscle biopsy of the vastus lateralis muscle (performed at the age of 46) stained with anti-human collagen VI antibodies. Visual inspection revealed decreased endomysial collagen VI staining. **A**: Control subject. **B**: TNX-deficient EDS patient. Bar = 50 µm; objective 10x.



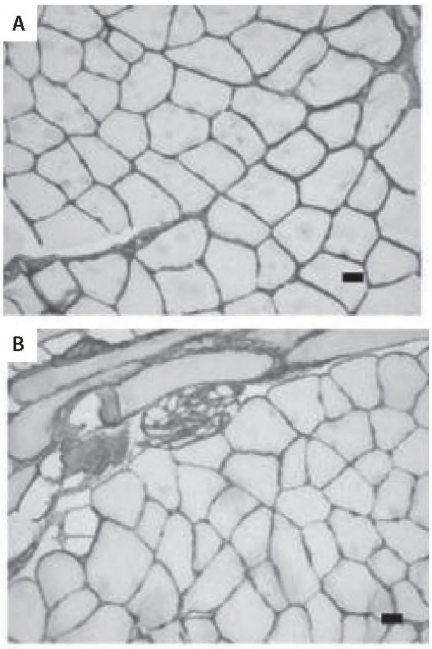
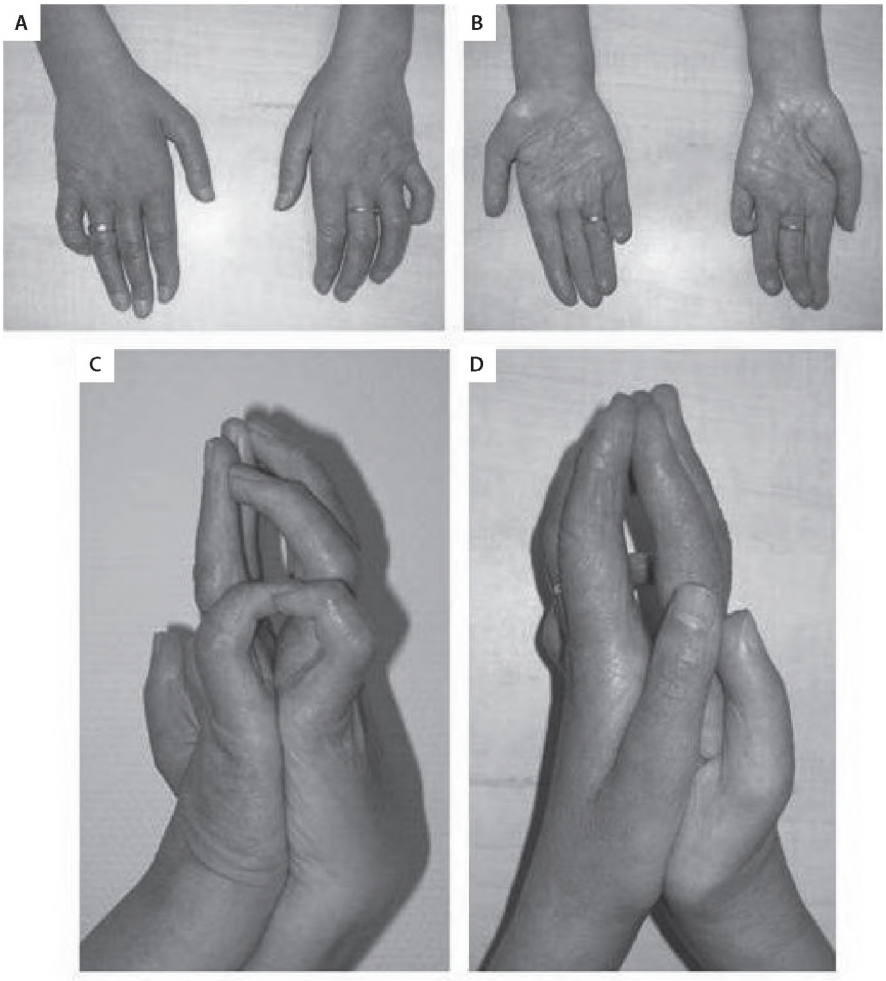


Figure 6 Muscle atrophy and contractures.

A and **B**: Muscle atrophy. **C** and **D**: Distal contractures of the fourth and fifth proximal and distal interphalangeal joints.



to fibrinogen. Its complex structure enables multiple interactions with other ECM (glyco) proteins, rendering TNX a crucial player in the organization of the ECM notably in skeletal muscle.[29-31] Upon disruption of the TNX gene, the expression of type VI collagen is significantly decreased.[32] Moreover, TNX and type VI collagen are collectively involved in collagen fibrillogenesis *in vitro* and *in vivo*.[33]

In short, where Kirschner *et al.* observed EDS-like symptoms in UCMD patients, we report on collagen VI myopathy-like symptoms in an EDS patient. The observations in this patient and the results of previous studies on TNX and collagen VI interaction provide support for a clinical and biochemical overlap between collagen VI-related myopathies and TNX-deficient type EDS (*Table 3*). This overlap, which was already proposed by Kirschner et al., is of conceptual interest and may in time have diagnostic and therapeutic consequences.[26] Further studies should elucidate whether muscle symptoms are common in this and other types of EDS.

Table 3 Comparison of clinical and biochemical findings in patients with TNX-deficient type of EDS, classical type of EDS, hypermobility type of EDS, and UCMD / Bethlem myopathy.

	TNX-deficient type EDS	**Classical type EDS**	**Hypermobility type EDS**	**UCMD / Bethlem myopathy**
Tendon/skeleton	Joint hypermobility	**Joint hypermobility**	**Generalized joint hypermobility,** recurrent joint dislocations, chronic joint / limb pain	Distal joint hypermobility, contractures, Kyphoscoliosis, Torticollis, prominent calcanei
Skin	Hyperextensible and smooth, velvety skin, minimal atrophic scars	**Skin hyperextensibility, widened atrophic scars**, smooth velvety skin, molluscoid pseudotumors, subcutaneous spheroids	**Skin involvement (hyperextensibility and/or smooth, velvety skin)**	Minimal atrophic scars, velvety skin, striae formation, keloid formation, follicular hyperkeratosis
Muscle	Generalized muscle weakness, hypotonia, distal contractures	Hypotonia, delayed gross motor development	Musculoskeletal pain	Mild to severe weakness, respiratory failure
Vasculature	Easy bruising	Easy bruising	Normal	Not reported
Other		Manifestations of tissue extensibility and fragility, surgical complications, fatigue		
Inheritance	Autosomal recessive	Autosomal dominant	Autosomal dominant	Autosomal dominant or autosomal recessive
ECM molecule	TNX	?, Collagen V	?, TNX	Collagen VI
Gene (locus)	*TNXB* (6p21.3)	?, *COL5* (9q34)	?, *TNXB* (6p21.3)	*COL6A1*, *COL6A2* (21q22.3), *COL6A3* (2q37)

EDS major criteria according to the revised classification (Villefranche) are printed in bold.[1] Characteristics of the TNX-deficient type EDS are according to Schalkwijk et al.[2] Zweers et al. demonstrated that TNXB haploinsufficiency is associated with the hypermobility type of EDS.[3] UCMD/BM myopathy characteristics were based upon the table of Kirschner et al.[26]

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
3. Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M, Kucharekova M, Boezeman JB, Schalkwijk J. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet 2003; 73: 214-217.
4. Beighton P. The Ehlers-Danlos syndromes. London: William Heineman Medical Books Limited; 1970.
5. Matsumoto K, Sawa H, Sato M, Orba Y, Nagashima K, Ariga H. Distribution of extracellular matrix tenascin-X in sciatic nerves. Acta Neuropathol (Berl) 2002; 104: 448-454.
6. Muellbacher W, Finsterer J, Mamoli B, Bittner RE, Trautinger F. Axonal polyneuropathy in Ehlers-Danlos syndrome. Muscle Nerve 1998; 21: 972-974.
7. Pretorius ME, Butler IJ. Neurologic manifestations of Ehlers-Danlos syndrome. Neurology 1983; 33: 1087-1089.
8. Peterson-Kendall F, Kendall-McCreary E, Geise-Provance P, McIntyre-Rodgers M, Romani WA. Muscles testing and Function with Posture and Pain. Baltimore, MD, USA: Lipincott Williams & Wilkins; 2005.
9. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
10. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. J Pathol 2003; 200: 423-428.
11. Galan E, Kousseff BG. Peripheral neuropathy in Ehlers-Danlos syndrome. Pediatr Neurol 1995; 12: 242-245.
12. Wenstrup RJ, Murad S, Pinnell SR. Ehlers-Danlos syndrome type VI: clinical manifestations of collagen lysyl hydroxylase deficiency. J Pediatr 1989; 115: 405-409.
13. Yis U, Dirik E, Chambaz C, Steinmann B, Giunta C. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord 2008; 18: 210-214.
14. Voermans NC, van Engelen BG. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord 2008; 18: 906.
15. Pinnell SR, Krane SM, Kenzora JE, Glimcher MJ. A heritable disorder of connective tissue. Hydroxylysine-deficient collagen disease. N Engl J Med 1972; 286: 1013-1020.
16. Hilderink BG, Brunner HG. Nevo syndrome. Clin Dysmorphol 1995; 4: 319-323.
17. Giunta C, Randolph A, Al-Gazali LI, Brunner HG, Kraenzlin ME, Steinmann B. Nevo syndrome is allelic to the kyphoscoliotic type of the Ehlers-Danlos syndrome (EDS VIA). Am J Med Genet A 2005; 133: 158-164.
18. Martina IS, van Koningsveld R, Schmitz PI, van der Meche FG, van Doorn PA. Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. European Inflammatory Neuropathy Cause and Treatment (INCAT) group. J Neurol Neurosurg Psychiatry 1998; 65: 743-747.
19. Pillen S, Verrips A, van Alfen N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. Neuromuscul Disord 2007; 17: 509-516.
20. Arts IM, Pillen S, Overeem S, Schelhaas HJ, Zwarts MJ. Rise and fall of skeletal muscle size over the entire life span. J Am Geriatr Soc 2007; 55: 1150-1152.
21. Salavoura K, Valari M, Kolialexi A, Mavrou A, Kitsiou S. A case of Ehlers Danlos syndrome type VI. Genet Couns 2006; 17: 291-294.
22. Farag TI, Schimke RN. Ehlers-Danlos syndrome: a new oculo-scoliotic type with associated polyneuropathy? Clin Genet 1989; 35: 121-124.
23. Takaluoma K, Hyry M, Lantto J, Sormunen R, Bank RA, Kivirikko KI, Myllyharju J, Soininen R. Tissue-specific changes in the hydroxylysine content and cross-links of collagens and alterations in fibril morphology in lysyl hydroxylase 1 knock-out mice. J Biol Chem 2007; 282: 6588-6596.
24. Voermans NC, Drost G, van Kampen A, Gabreels-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG. Recurrent neuropathy associated with Ehlers-Danlos syndrome. J Neurol 2006; 253: 670-671.

25. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
26. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, Anton-Lamprecht I, Muntoni F, Hanefeld F, Bonnemann CG. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A 2005; 132: 296-301.
27. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
28. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.
29. Lethias C, Carisey A, Comte J, Cluzel C, Exposito JY. A model of tenascin-X integration within the collagenous network. FEBS Lett 2006; 580: 6281-6285.
30. Matsumoto K, Saga Y, Ikemura T, Sakakura T, Chiquet-Ehrismann R. The distribution of tenascin-X is distinct and often reciprocal to that of tenascin-C. J Cell Biol 1994; 125: 483-493.
31. Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. Dev Dyn 1995; 203: 491-504.
32. Minamitani T, Ariga H, Matsumoto K. Deficiency of tenascin-X causes a decrease in the level of expression of type VI collagen. Exp Cell Res 2004; 297: 49-60.
33. Minamitani T, Ikuta T, Saito Y, Takebe G, Sato M, Sawa H, Nishimura T, Nakamura F, Takahashi K, Ariga H, Matsumoto K. Modulation of collagen fibrillogenesis by tenascin-X and type VI collagen. Exp Cell Res 2004; 298: 305-315.

CHAPTER 4

Neuromuscular involvement in various types of Ehlers-Danlos syndrome

Adapted from:
Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij IA, Hamel BC, van Engelen BG.
Ann Neurol. 2009;65:687-97.

Abstract

The Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Muscle involvement is plausible based on recently discovered interactions between muscle and extracellular matrix molecules; however, muscle symptoms are only sporadically reported in case reports. Therefore, we designed a cross-sectional study to find out whether neuromuscular features are part of the EDS phenotype.

Standardized questionnaires, physical examination, laboratory investigations, nerve conduction studies, electromyography, muscle ultrasound, and muscle biopsy were performed in 40 EDS patients with the vascular, classical, tenascin-X (TNX)-deficient type EDS, and hypermobility type of EDS due to *TNXB* haploinsufficiency.

Muscle weakness, myalgia, and easy fatigability were reported by the majority of patients. Mild to moderate muscle weakness (85%) and mild reduction of vibration sense (60%) were common. Nerve conduction studies revealed axonal polyneuropathy in five patients (13%). Needle electromyography showed predominantly myopathic features in nine patients (26%), and a mixed neurogenic-myopathic pattern in most (60%). Muscle ultrasound revealed increased echo intensity (48%) and atrophy (50%). Mild myopathic features were seen on muscle biopsy of five patients (28%). Overall, patients with the hypermobility type EDS due to *TNXB* haploinsufficiency were least affected.

Mild to moderate neuromuscular involvement is commonly present in various types of EDS, with a remarkable relation between residual TNX level and degree of neuromuscular involvement, compatible with a dose-effect relationship. The findings of this study should increase awareness of neuromuscular symptoms and signs in EDS patients and improve clinical care. It also points to a possible role of the extracellular matrix in muscle and peripheral nerve function.

Introduction

The Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders (ICTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. The revised classification of EDS in six major types is based upon clinical and biochemical features, and consists of major and minor diagnostic criteria (*Table 1; Figure 1*).[1] The hypermobility type is the most common type of EDS, followed by the classical type. Together, these types account for approximately 90% of all cases.[2] The vascular type is far less common, but associated with development of vascular dissections and aneurysms, which may cause severe neurological complications. The kyphoscoliotic, arthrochalasia, and dermatosparaxis types are rare, as are the various other forms. In 2001, a new autosomal recessive type of EDS caused by deficiency of tenascin-X (TNX) was identified.[3,4] Subsequently, *TNXB* haplo-insufficiency was found to be associated with the hypermobility type of EDS in a minority of patients.[5,6]

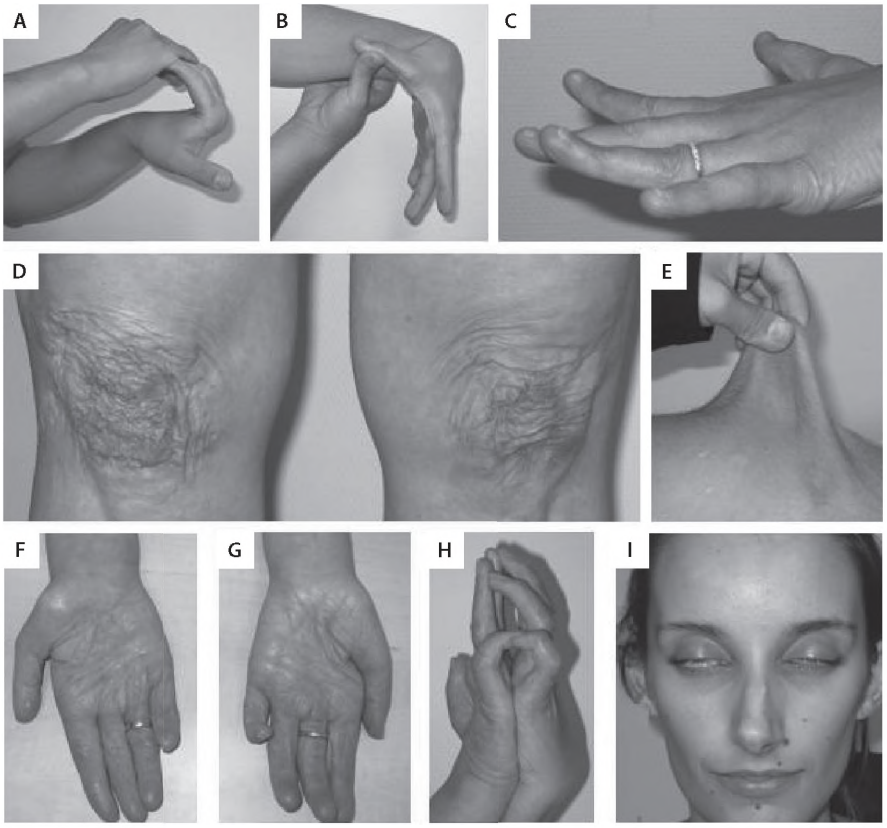
Muscle involvement can be expected based on interactions between muscle and extracellular matrix (ECM) molecules.[7] Furthermore, muscle hypotonia and muscle rupture are part of the diagnostic criteria of EDS, and fatigue, musculoskeletal pain, and delayed gross motor development are described as associated features (*Table 1*).[1] However, muscle symptoms such as muscle weakness and exercise intolerance are only sporadically reported in case reports. They are generally interpreted to result from increased distensibility of tendons, or from exercise avoidance due to joint hypermobility.[1,2,8,9] Within this frame of reference, symptoms of mild muscle weakness might not easily be detected as such. Furthermore, mild muscle weakness can also manifest as fatigue or compensatory musculoskeletal pain in non-affected muscles.[1] Furthermore, patients with EDS are generally not seen by a neurologist, and therefore mild neuromuscular signs may also remain unnoticed.

Only two previous studies have directly investigated neuromuscular involvement in EDS. In a study of muscle function in a patient with classical type EDS, muscle weakness and reduced joint proprioception were found.[10] In a recent case study we demonstrated muscle weakness in two TNX-deficient EDS patients, which most likely resulted from abnormal muscle ECM composition and impaired myofascial force transmission.[11]

To find out whether neuromuscular features are in fact part of EDS, we performed a cross-sectional observational study on the occurrence of neuromuscular symptoms in various EDS types. Patients with the classical, the vascular, and the TNX-deficient type EDS were included, since clinical criteria for these types are most stringent, and the diagnosis can often be confirmed by biochemical or genetic analysis. To study the influence of residual TNX levels on neuromuscular involvement, we also included patients with the hypermobility type of EDS with reduced TNX serum levels due to *TNXB* haploinsufficiency.[5]

Figure 1 Clinical features of EDS.

A and **B**: Joint hypermobility of hands in a patient with the TNX-deficient type EDS. **C:** Joint hypermobility of hands in a patient with the classical type EDS. **D:** Atrophic scars in a patient with the classical type EDS. **E:** Skin hyperextensibility in a patient with the TNX-deficient type EDS. **F**, **G** and **H**: Contractures of the distal phalanges of digitum IV and V of both hands and atrophy of intrinsic hand muscles in a patient with the TNX-deficient type EDS, previously reported by Voermans et al.[12] **I:** Incomplete eye closure in a patient with the vascular type EDS.



Methods

Study population

A total of 40 patients of four well-defined EDS types was included to investigate whether findings are related to specific types of EDS. We recruited ten patients of each type. Patients with the classical and vascular types were recruited among members of the Dutch EDS patient organization (www.ved.nl) without knowledge of their neuromuscular status. These patients all fulfilled the clinical criteria.[1] Results of biochemical and genetic analysis which had been performed elsewhere were noted. The patients with TNX-deficient type EDS were referred to us by the dermatology, human genetics or internal medicine departments of our hospital; these 10 patients represent all adult Dutch patients with this type of EDS diagnosed so far, and dermatological, genetical, and biochemical features of six of them have previously been described by Schalkwijk et al.[3] Measurement of TNX in serum (n = 10) and mutation analysis of *TNXB* (n = 6) had previously been performed, and the results of these tests were noted.[3,5] Ten family members with reduced serum levels of TNX due to *TNXB* haplo-insufficiency and clinical features of the hypermobility type EDS were recruited without knowledge of their neuromuscular status. Their dermatological and biochemical features were previously described by Zweers et al.[5] For comparison, a number of healthy age and sex-matched controls, mostly hospital employees and their family members, were asked to fill out the symptoms questionnaire. The study was performed in accordance with the Helsinki criteria after approval by the local ethics committee.[27] Informed consent was obtained from all patients.

Clinical studies

Standardized questionnaires

All patients completed a brief questionnaire focusing on medical history, muscle symptoms i.e. muscle weakness, muscle hypotonia, presence of frequent or continuous myalgia, increased fatigability compared to peers, use of a wheelchair, use of walking aids, walking distance, and performing sports. In addition, patients and subjects were asked for occurrence of paresthesias or numbness to detect entrapment neuropathies.

Standardized physical examination

The patients were all examined by one of the authors using a standardized protocol. Hypermobility was scored with the Beighton score.[28] Skin hyperextensibility was tested on the extensor side of the forearm, and classified as normal or increased (> 4 cm).[1] Easy bruising was defined as presence of spontaneous ecchymoses, which frequently recur in the same area and cause characteristic brownish discoloration.[1] Cranial nerves were tested. Muscle strength was graded according to the Medical Research Council (MRC) system.[29] Thirteen

Table 1 Various types of Ehlers-Danlos syndrome. Diagnostic criteria and literature review on neuromuscular involvement.

Diagnostic criteria			
EDS type	*Mode of inheritance* *Biochemical / genetic defect*	*Major criteria*	*Minor criteria and associated neuromuscular features*
Classical type	AD Structural defects in the proα1(V) and proα2(V) chains of collagen V encoded by *COL5A1* in approximately half of the patients	Skin hyperextensibility Widened atrophic scars (manifestation of tissue fragility) Joint hypermobility	Muscle hypotonia Fatigue
Hypermobility type	AD / AR Unknown Reduced serum level of TNX / *TNXB* haplo-insufficiency in few patients (< 5%)	Skin involvement (hyperextensibility and / or smooth, velvety skin) Generalized joint hypermobility	Musculoskeletal pain
Vascular type	AD Structural defects in the proα1(III) chain of collagen type III encoded by *COL3A1* in approximately half of the patients	Thin, translucent skin Arterial / intestinal / uterine fragility or rupture Extensive bruising Characteristic facial appearance	Muscle rupture
Other types	Various	Various	Muscle hypotonia (in arthrochalasia type and kyphoscoliotic type) Delayed gross motor development (in kyphoscoliotic type)
TNX-deficient type	AR Deficiency of TNX, caused by mutations in *TNXB*	Generalized joint hypermobility Skin hyperextensibility Easy bruising without atrophic scarring.	

Mode of inheritance, biochemical and/or genetic defect, diagnostic criteria, and muscle and peripheral nerve involvement reported in literature. (AD: autosomal dominant; AR; autosomal recessive). The literature review was performed in Pubmed with the following search terms: "Ehlers-Danlos syndrome / EDS" and "muscle / myopathy" or "peripheral nerve / polyneuropathy". Subsequently, we checked the references of these articles for relevant other articles.

Literature review	
Peripheral nerve involvement	*Muscle involvement*
Polyneuropathy[13]	Muscle hypotonia and hypoplasia[9] Muscle weakness [10] Fatigue[1]
(Recurrent) neuropathy[14-16]	Muscle cramps and myalgia[1,17]
Brachial plexopathy[18] Pressure neuropathy caused by arterial rupture[19]	Muscle cramps, myalgia, finger flexor contractures[20]
(Axonal) polyneuropathy[21,22] HNPP[23]	Muscle cramps[8] Muscular dystrophy[24] Muscle hypotonia[25,26] Delayed motor development[26]
	Muscle weakness[11,12]

proximal and distal limb muscle groups bilaterally and two trunk muscle groups were tested, which resulted in a maximum MRC sum score of 140. Muscle strength was defined as mildly reduced when the MRC sum score was 126 – 139 (mean MRC 4.5 - < 5); moderately reduced in case of a MRC sum score of 112 - 125 (mean MRC 4 - < 4.5); and severely reduced with a MRC sum score below 112 (mean MRC < 4). To verify presence of mild muscle weakness, muscle force was also measured with dynamometry performed in five muscle groups bilaterally in standardized positions using a CIT hand held dynamometer (www.citec.nu). The median of three measurements of each muscle group on both sides was calculated and compared with the median and p5 value of healthy controls previously reported.[30] Muscle tone of the biceps brachii and quadriceps muscles was evaluated bilaterally and semi-quantitatively in a sitting position (0 = hypotonia; 1 = normotonia; muscle tone sum score 0 - 4), with hypotonia defined as a sum score < 4. Coordination was tested bilaterally with use of finger-nose-finger test and heel-to-knee-to-toe test, and tandem gait (0 = > 2 cm deviation or > 2 steps to the side; 1 = ≤ 2 cm deviation or ≤ 2 steps to the side; 2 = normal; coordination sum score 0-10). Reduced coordination was defined as a sum score < 10. Testing of sensation consisted of assessment of vibration sense (with Rydell-Syffer tuning fork),[31] pain sense, and touch sense in legs and arms both proximally and distally; movement sense was tested in distal legs and arms (0 = absent; 1 = reduced, and 2 = normal). The Romberg test was scored as following: not able to stand with eyes closed = 0; standing with eyes closed < 60 seconds = 2; 60 seconds standing with eyes closed = 4). The sensation and Romberg scores cumulated in a sensation sum score of 0 - 60, with reduced sensation defined as a sum score < 60. Reflexes were evaluated bilaterally on a 3-point scale (0 = absent; 1 = reduced; 2 = normal, with a reflex sum score of 0 – 16), with hyporeflexia defined as a sum score < 16.

Functional measurements included the Vignos scale (measures lower extremity function; 1 = being able to walk and climb stairs without assistance; 10 = confined to bed),[32] Brooke scale (measures upper extremity function; 1 = patients being able to fully abduct their arms; 6 = no useful function of hands),[33] Rivermead Mobility Index (measures the ability to move its own body; 0 = severely dependent on others for mobility; 15 = normal),[34] and Modified Rankin Scale (measures recovery of motor function after stroke; 0 = normal; 5 = severe disability; 6 = dead).[35,36]

Ancillary investigations

Laboratory investigation

Creatine kinase (CK) was measured in the laboratory of our centre (normal values: CK < 220 U/l (males) and CK < 170 U/l (females)). In patients in whom a polyneuropathy was detected, ancillary laboratory investigations were performed to exclude an underlying cause of polyneuropathy according to the Dutch national multidisciplinary protocol (vitamin B12, glucose, thyroid function, calcium, phosphate, and liver function).

Clinical neurophysiology

Electrophysiological examination was performed using standard techniques with a Medelec Synergy EMG system (Viasys, Oxford Medical Instruments, Surrey, United Kingdom). Both nerve conduction studies and needle electromyography were performed according to standardized clinical protocols for the detection of a polyneuropathy and myopathy. Findings were compared with normal values from a previously established database in our centre.

Nerve conduction studies

Nerve conduction studies (NCS) included compound muscle action potential (CMAP) amplitudes and nerve conduction velocities (NCV) of the peroneal and tibial nerves, sensory nerve action potential (SNAP) amplitude and NCV of the sural nerve (if abnormal bilateral measurements were performed), and H-reflexes of the tibial nerves. In case of (suspicion of) a polyneuropathy, NCV and CMAP of the median nerve, and NCV and SNAP of the radial nerve were also measured. Polyneuropathy was defined according to the guidelines of the American Association of Electrodiagnostic Medicine.[37] We did not systematically screen for entrapment neuropathies.

Needle electromyography

Needle electromyography was performed of the tibialis anterior, rectus femoris, biceps brachii, deltoid, and paraspinal L4-L5 muscles, all on the left side. Insertional activity, recruitment pattern on voluntary contraction, and motor unit action potential characteristics (amplitude, duration, number of phases) were noted. Motor Unit Action Potential (MUAP) characteristics and recruitment pattern were defined as follows: 'predominantly myopathic' (small (3 - 8 ms), polyphasic units with moderate to normal recruitment); 'predominantly neurogenic' (large (> 12 ms) units with poor to moderate recruitment), or 'mixed myopathic - neurogenic', all in at least two of the muscles tested. Turns and amplitudes measurements of the rectus femoris and biceps brachii muscles were performed when myopathic MUAPs were seen from routine needle examination in these muscles.

Muscle Ultrasound

Muscle ultrasound examinations were performed bilaterally in five muscles (biceps brachii muscle, rectus femoris, and tibialis anterior muscles and extensors and flexors in forearm) using a standard technique as described previously.[38,39] A broadband linear 5 - 17 MHz transducer (Philips IU22, The Netherlands) was used. All system-setting parameters were kept constant throughout the study (gain 70 dB, compression 55, no adjustments in time gain compensation or focus).

For echo-intensities (EI), a region of interest was selected in each image using the lasso function. The criteria for selecting this region were inclusion of as much of the muscle as possible without surrounding fascia. Mean echo intensity of this region was then calculated

with a standard histogram function (Adobe Photoshop; Adobe systems Inc., San Jose, California, USA) and expressed as a value between 0 (black) and 255 (white) as the ultrasound was created with 8 bit grey scale. Three consecutive measurements were taken of every muscle and results were averaged.[38] Muscle thickness was measured with electronic callipers. Echo intensity and muscle thickness are different for each muscle group, and depend on age and sex.[39,40] We used a database previously established in our centre to calculate normal values for each muscle corrected for age and sex (unpublished data).[39,40] In order to compare individual patients and muscles in this study, echo-intensity and muscle thickness were transformed into Z-scores.[39,40] In effect, the Z-score reflects the number of standard deviations a measure deviates from normal, given a certain age and sex. Abnormal EI was defined as one muscle with Z-score > 2. For muscle atrophy, the same cut-off value was used (Z-score < -2).

Muscle Biopsies

Muscle biopsy specimens were obtained from the right vastus lateralis muscle. Frozen sections of 10 µm were examined under a light microscope with Hematoxylin-Phloxine, Periodic acid-Schiff, Sudan Black B, Trichrome-Gomori and enzyme histochemical staining (ATPase (pH 4.2, 4.6 and 10.3), succinic dehydrogenase, reduced nicotinamide adenine dinucleotide-tetrazolium reductase, cytochrome C oxidase, acid phosphatase). Collagen VI staining was performed with monoclonal mice anti-human collagen VI antibodies (Chemicon, Millipore, Billerica, MA, USA). Additionally, we investigated six muscle samples (of one patient of each type, and two additional biopsies of patients with the classical type) with electron microscopy. For that purpose specimens were fixed in glutaraldehyde, postfixed in Osmium Tetroxide, embedded in Epon and stained with uranlyacetate and lead citrate. We used control specimens previously obtained from patients without clinical or morphological signs of myopathy. Muscle biopsy specimens were assessed by one investigator.

Statistics

The demographic and clinical data were recorded as variables in a SPSS database. Statistical analysis was performed using SPSS version 14.0 (SPSS Inc, Chicago, IL, USA). In addition to the analysis of the data of all EDS patients, we performed subgroup analysis to compare the various types of EDS. A Chi-square or Fisher's exact test was used for dichotomous variables. With continuous variables, a Student's t-test was used in case of a normal distribution, and a Mann-Whitney-U test in case of a non-parametric distribution. Correlation was calculated with Pearson correlation coefficient. Statistical significance was defined as a p-value below 0.05 (two-sided).

Definition of neuromuscular involvement

Neuromuscular involvement was defined as consistent abnormal findings on questionnaires or physical examination or both, supported by abnormal results of appropriate ancillary investigations.

Results

Study population

A total of 40 patients from 27 families was included in this study: 10 non-related classical type EDS patients (eight female; median age 36; age range 27-63), 10 non-related vascular type patients (seven female; median age 33; age range 14 - 41), 10 TNX-deficient type patients (seven female; median age 42; age range 20 - 60), and 10 hypermobility type due to *TNXB* haplo-insufficiency (nine female; median age 30; age range 23 - 62); the latter two groups belonging to seven families.

The diagnosis was based on the diagnostic criteria in all vascular and classical type EDS patients.[1,3] Biochemical analysis was previously performed in five patients with the classical type EDS and revealed reduced amount of collagen V in two patients. All patients with the vascular type EDS had reduced expression of collagen III. This diagnosis was genetically confirmed in three vascular type patients: the mutations found in *COL3A1* were: 3512 G>A (n = 1); 545 G>C (n = 1); and 370 G>T (n = 1). Mutation analysis was not performed in the other seven vascular type EDS patients. The clinical features of all TNX-deficient type EDS patients were similar to those previously described.[3,4] TNX in serum was < 0.1 ng/ml (n = 10), and mutation analysis performed in six patients revealed the following mutations in *TNXB*: homozygous 2 bp deletion in exon 8 [AA56063] del (n = 2); homozygous 30 kB deletion (n = 1); heterozygous 30 kB deletion and second TNX mutation not found (n = 3) (data previously published by Schalkwijk et al, 2001).[3] Four of the first-grade family members of the TNX-deficient type EDS patients had been diagnosed with hypermobility type EDS; the other six subjects had not been diagnosed with the hypermobility type so far, but did reveal one or more of the clinical features of the hypermobility type of EDS.[1,5] TNX in serum was found to be half of the normal value in all subjects (range 46 – 68% of normal) (n = 10).[5] Mutation analysis had revealed *TNXB* haploinsufficiency in nine of them: heterozygous 2 bp insertion in exon 3 [GT44906] ins (n = 6; all relatives of the index patient); heterozygous for 30 kB deletion (n = 1); heterozygous 2 bp deletion in exon 8 [AA56063] del (n = 2). In one patient, no mutation was found (daughter of the patient in whom the second *TNXB* mutation was not found) (data previously published by Zweers et al, 2003).[5]

Twenty healthy subjects filled out the symptoms questionnaire (14 female; median age 35; age range 22 - 64).

Clinical studies

Standardized questionnaires

All patients completed the questionnaires. Seven EDS patients reported a previous neurological disease, most frequently among the patients with the vascular type of EDS: carotid-cavernous fistula, migraine, brachial plexopathy due to subclavian artery aneurysm, subarachnoidal haemorrhage, and young stroke. In two of them, the neurological event was the initial symptom of the vascular type of EDS. Furthermore, two patients with the classical type of EDS reported scoliosis with lumbar stenosis and carpal tunnel syndrome respectively, and one patient with the TNX-deficient type reported carpal tunnel syndrome. No other complaints suggestive for entrapment neuropathies were reported.

Results of questionnaires are listed in *Table 2*. Overall, patients with the classical type reported most complaints on the neuromuscular questionnaire.

Standardized physical examination

Physical examination was performed in all patients and revealed typical EDS features (joint hypermobility, skin hyperextensibility, and easy bruising) as described in the clinical criteria (*Figure 1*).[1,3,5] One vascular type EDS patient had mild facial weakness and incomplete eye closure (*Figure 1*), without clinical signs of myasthenia gravis or myotonia. Another vascular type EDS patient had a partial left oculomotor paresis after surgical correction of a left caroticocavernous fistula. One patient with the TNX-deficient type EDS had generalized muscle weakness, contractures of the distal phalanges of digits IV and V of both hands, and atrophy of intrinsic hand muscles (*Figure 1*)(previously reported by Voermans et al).[11,12]

Results of physical examination are listed in *Table 3*. Mild to moderate muscle weakness (mean MRC 4 - 5) was found in all types of EDS. Muscle weakness tended to be more prominent in proximal limb muscles and axial muscles: knee flexion, hip flexion, neck and trunk flexion, and hip extension (mean MRC < 4.4); whereas all other muscles tested had a mean MRC of > 4.4 (data not shown). MRC sumscore was not correlated statistically significantly with age (r = - 0.17; n.s.). Results of dynamometry corroborated the findings of manual muscle testing: the median force of all 5 muscles tested bilaterally (measured in Newton) of all EDS patients was below or at the p5 of the normal values (data not shown).

Hypotonia was present in 43% of the EDS patients (n = 17/40), and coordination was reduced in 18% (n = 7/40). Sensory testing was abnormal in 60% of the patients (n = 24/40); this predominantly consisted of mild reduction of vibration sense in legs. Functional assessment showed mild impairment in the vascular, classic, and TNX-deficient type of EDS, but not in patients with the hypermobility type EDS due to *TNXB* haploinsufficiency.

Table 2 Results of questionnaires. Number (%) of EDS patients and controls who report various neuromuscular symptoms, and p-value of difference in frequency of reported symptoms between EDS patients and controls.

	Vasc n = 10	Clas n = 10	TNXd n = 10	TNXh n = 10	Differences between EDS types P-value (#)		EDS total n = 40		Controls n = 20		Differences patients-controls P-value (#)
Muscle weakness	6	9	9	2	0.005	clas – TNXh TNXd - TNXh	26	65%	0		< 0.001
Muscle hypotonia	2	8	5	2	0.023	clas – vasc clas – TNXh	17	43%	0		0.001
Myalgia (continuously or frequently after exercise)	6	10	9	4	0.011	clas – TNXh	29	73%	2	10%	< 0.001
Easy fatigability (compared to peers)	6	9	7	2	0.005	clas – TNXh	24	60%	0		< 0.001
(Intermittent) paresthesias in arms and / or legs	4	6	3	0	0.011	clas – TNXh	13	33%	1	5%	0.018
Walking distance < 5 km	5	9	6	2	0.005	clas – TNXh	22	55%	1	5%	0.001
(Intermittent) use of wheelchair	1	2	0	0	n.s.		3	8%	0		n.s.
Use of walking aids	2	5	4	0	n.s.		11	28%	0		< 0.001
Performing sports (at least once a week)	5	5	5	5	n.s.		20	50%	14	70%	n.s.

Vasc: vascular type EDS; Clas: classical type EDS; TNXd: TNX-deficient type EDS; TNXh: hypermobility type EDS due to *TNXB* haploinsufficiency. (#) Chi-square test; n.s. no statistically significant differences.

Ancillary investigations

Results of ancillary investigations are listed in *Table 3*.

Laboratory investigations

Creatine kinase was normal in most patients and was not correlated statistically significantly with age (r = -0.13; n.s.). Ancillary laboratory investigations revealed no metabolic abnormalities in patients with a polyneuropathy (data not shown). Screening for vasculitis as a cause of the polyneuropathy was not performed in these patients since systemic signs of vasculitis were absent.

Clinical neurophysiology

Nerve conduction studies were performed in 39 patients, and needle electromyography in 35 patients.

Nerve conduction studies

Nerve conduction studies were abnormal in 38% of the patients (n = 15/39), fulfilling the criteria of a sensorimotor axonal polyneuropathy in 13% (n = 5/39). Low CMAP amplitudes in the legs with normal sensory conduction were found in 21% of the patients (n = 8/39), predominantly in patients with myopathic MUAPs and a myopathic recruitment pattern on electromyography (see below). This might suggest that CMAP amplitudes were low secondary to myopathy.

Needle electromyography

Needle electromyography was abnormal in 91% of all EDS patients (n = 32/35), and no statistically significant differences existed between the EDS types. The recruitment pattern and MUAP characteristics were predominantly myopathic in 26% of the patients (n = 9/35), predominantly neurogenic in two patients with the TNX-deficient type with a polyneuropathy, and a mixed pattern was encountered in 60% of all EDS patients (n = 21/35). Complex repetitive discharges were detected in one muscle in two patients; no other signs of spontaneous activity were found.

Muscle Ultrasound

Muscle ultrasound was performed in all patients and showed increased echo intensities in 48% (n = 19/40) and atrophy in 50% of the patients (n = 20/40). Increased echo intensity was most pronounced in the flexors and extensors in the forearm and in the tibialis anterior muscle, and atrophy was most pronounced in the quadriceps muscle. In one patient with the vascular type EDS, an area of increased signal intensity with a diameter of 1 cm in the right quadriceps was observed, probably reflecting a previous haemorrhage which might have been due to local muscle rupture.

Table 3 Results of physical examination and ancillary investigations. Results of testing of muscle force, muscle tone, coordination, sensation and reflexes: the mean MRC sum score, and the number of patients with abnormal test results are shown. Results of ancillary investigations: the median creatine kinase level with range; the number of patients in which nerve conduction, electromyography, muscle ultrasound, and muscle biopsy was performed (bold), and the number of patients in which the test results were abnormal are shown.

	Vasc	**Clas**	**TNXd**	**TNXh**	**EDS total**	**Differences between EDS types P-value**		
Physical examination	*n = 10*	*n = 10*	*n = 10*	*n = 10*	*n = 40*			
Mean MRC sum score (0-140) (SD)	128.9 (11.4)	124.8 (8.3)	127.6 (9.2)	135.8 (3.2)	127 (13.8)	0.002 0.021	clas - TNXh TNXd - TNXh	(§)
Normal muscle strength	2	0	2	2	6	n.s.		(*)
Mild muscle weakness	5	5	4	8	22	n.s.		(*)
Moderate muscle weakness	2	4	4	0	10	n.s.		(*)
Severe muscle weakness	1	1	0	0	2	n.s.		(*)
Hypotonia	2	7	7	1	17	0.020	clas - TNXh TNXd - TNXh	(*)
Reduced coordination	1	3	3	0	7	n.s.		(*)
Reduced sensation	6	7	6	5	24	n.s.		(*)
Hyporeflexia	1	3	6	0	10	0.011	TNXd -TNXh	(*)
Vignos (1-10)	1.9	2.0	1.5	1.0	1.6	0.032	clas - TNXh	(§)
Brooke (1-6)	1.0	1.2	1.1	1.0	1.1	n.s.		(§)
Rivermead (0-15)	13.1	12.5	14.5	15.0	13.8	0.002	clas - TNXh	(§)
Modified Rankin scale (0-6)	1.7	2.0	1.3	0.2	1.1	0.006 0.002 < 0.001	vasc - TNXh clas - TNXh TNXd - TNXh	(§)
Laboratory investigations	*n = 8*	*n = 9*	*n = 10*	*n = 6*	*n = 33*			
Median creatine kinase (U/l) (range)	130 (47-681)	119 (40-169)	107 (50-287)	97 (57-223)	118 (40-681)	n.s.		(±)
Number of patients with increased CK	2	0	1	1	4	n.s.		(*)
Nerve conduction studies	*n = 9*	*n = 10*	*n = 10*	*n = 10*	*n = 39*			
Abnormal	4	2	8	1	15	0.023 0.006	vasc - TNXd TNXd - TNXh	(*)
Axonal sensomotor polyneuropathy	1	0	4	0	5	n.s.		(*)

Table 3 Continued.

Low CMAP amplitudes in legs	2	1	4	1	8	n.s.	(*)
Entrapment neuropathy :							
CTS		1	1[1]	0	2	n.s.	(*)
Peroneal entrapment	1				1	n.s.	(*)
Electromyography	*n = 9*	*n = 10*	*n = 7*	*n = 9*	*n = 35*		
Abnormal	8	9	7	8	32	n.s.	(*)
MUAP characteristics and recruitment pattern:							
Predominantly myopathic	3	1	1	4	9	n.s.	(*)
Predominantly neurogenic	0	0	2	0	2	n.s.	(*)
Mixed myopathic - neurogenic	5	8	4	4	21	n.s.	(*)
Myopathic pattern in T/A							
Rectus femoris	4/5	3/7	0/5	3/9	10/26		
Biceps brachii	6/9	3/8	2/6	2/8	13/31		
Muscle ultrasound	*n = 10*	*n = 10*	*n = 10*	*n = 10*	*n = 40*		
Increased echo intensity	6	7	4	2	19	n.s	(*)
Reduced muscle diameter	6	5	6	3	20	n.s	(*)
Muscle biopsy	*n = 3*	*n = 8*	*n = 5*	*n = 3*	*n = 18*		
Mild myopathic features	2	1	1	1	5	n.s.	(*)

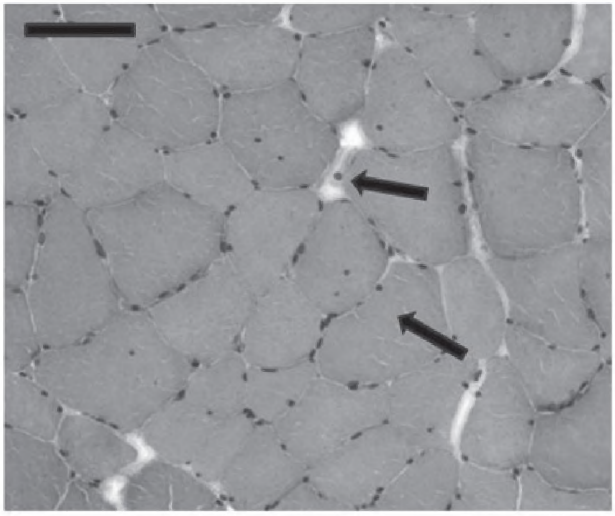
Vasc: vascular type EDS; Clas: classical type EDS; TNXd: TNX-deficient type EDS; TNXh: hypermobility type EDS due to *TNXB* haploinsufficiency. [1]In the patient who also had low CMAPs in legs. (§) Student's t-test; (*) Fisher's exact test; (±) Mann-Whitney-U test; n.s. no statistically significant differences. A Chi-square or Fisher's exact test was used for dichotomous variables. With continuous variables, a Student's t-test was used in case of a normal distribution, and a Mann-Whitney-U test in case of a non-parametric distribution.

Muscle Biopsies

Needle muscle biopsy was performed in 18 patients and wound healing was normal in all. Findings of histological analysis of muscle biopsies were slightly abnormal in 28% of the patients (n = 5/18), found at least once in every EDS type. These included increased variation of fibre diameter, sporadic isolated atrophic fibres, a mild increase of internal nuclei *(Figure 2)*, or the presence of some lobulated fibres. In one patient one ragged red fibre was seen (female 50 years), and in another case few COX-negative fibres were found (male, 37 years), but this might also be present in healthy subjects. Necrotic fibres, and signs of fibrosis or fibre

Figure 2 Mild myopathic changes in the muscle biopsy (lateral vastus muscle) of a hypermobility type EDS patient due to *TNXB* haploinsufficiency.

Mildly increased variation in fibre diameter and increase of central nuclei (5%)(arrow). Bar = 100 µm.



type grouping were absent. Histochemical and enzymehistochemical staining showed no signs of congenital myopathy or dystrophy. Collagen VI staining was present in both endo- and perimysium, and the distribution pattern and intensity of staining did not differ from control sections. No vascular abnormalities, such as perivascular infiltrations, perivascular fibrosis or abnormal vessel calibres were present.

We investigated six muscle samples (of one patient of each type, and two additional biopsies of patients of the classical type) with electron microscopy (EM). Overall, qualitative evaluation of the EM images showed relatively low connective tissue density; however, this varied between the histological location within the biopsy specimen (endomysium between individual muscle fibres; endomysium between muscle fibre and capillaries; perimysium at the septa). Furthermore, in the biopsy of a patient with the classical type EDS, short collagen fragments were seen at the sarcolemma (*Figure 3*). This finding was confirmed in the biopsies of a second and third patient with the classical type EDS.

A summary of the findings of clinical and ancillary investigations is presented in *Table 4*; this reflects a variable degree of neuromuscular involvement in the vascular, classical, and TNX-deficient EDS types, and to a lesser extent in patients with the hypermobility type EDS due to *TNXB* haploinsufficiency.

Figure 3 Electron microscopy of the biopsy of a classical type EDS patient (**A**) and a control subject (**B**).

A lower density of collagen fibrils in the ECM between muscle fibres (long arrow) and between muscle fibres and capillaries (C) (short arrow) in the biopsy of the classical type EDS patient (**A**) than in a control biopsy (**B**). Furthermore, collagen fibrils are more often short in the biopsy of the classical type EDS patient (**A**) than in a control biopsy (**B**). Bar = 1 µm.

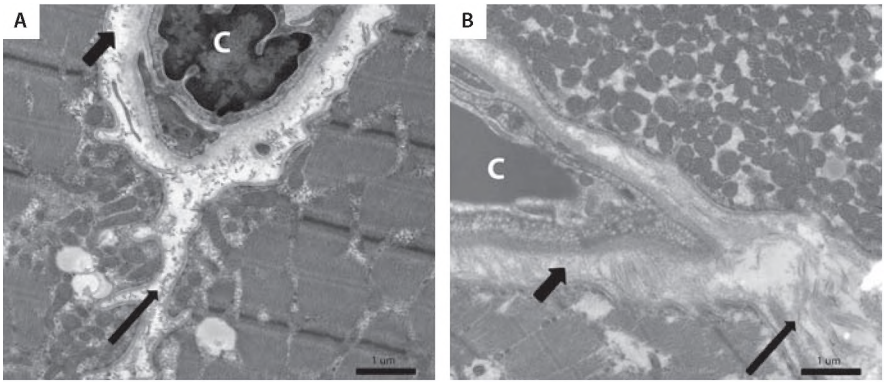


Table 4 Summary of findings: Neuromuscular involvement in EDS.

	Vasc	Clas	TNXd	TNXh
Questionnaires				
Neuromuscular complaints	+	+++	++	+/-
Physical examination				
Reduced sensation	+	+	+	+/-
Muscle weakness	+	+	+	+/-
Functional impairment	+	+	+	-
Clinical neurophysiological studies				
Polyneuropathy (NCS)	+	-	++	-
Abnormal MUAPs (myopathic or mixed myopathic-neurogenic (EMG))	++	+	+	+
Muscle ultrasound				
Increased echo intensity	+	+	+	+/-
Muscle biopsy				
Myopathic changes	+	+/-	+/-	+/-

- : none; +/- : mild in part of patients; + : mild; ++ : moderate; +++ : severe. Vasc: vascular type EDS; Clas: classical type EDS; TNXd: TNX-deficient type EDS; TNXh: hypermobility type EDS due to *TNXB* haploinsufficiency.

Discussion

This cross-sectional observational study demonstrates mild to moderate neuromuscular involvement in a large proportion of EDS patients. To our knowledge, this is the first systematic study on neuromuscular involvement in various well defined EDS types. This involvement consisted of an axonal sensorimotor polyneuropathy predominantly in the TNX-deficient type, whereas patients of all types had either mixed myopathic-neurogenic or myopathic features on electromyography. However, the muscle biopsy revealed only mild changes. Furthermore, complete absence of TNX (TNX-deficient type EDS) was associated with more severe neuromuscular symptoms than reduction of TNX serum levels (hypermobility type EDS due to *TNXB* haploinsufficiency). An inverse relation between residual TNX levels and degree of neuromuscular involvement was suggested by the differences between these two EDS types.

The results of this study confirm the findings of previous case reports and animal studies on neuromuscular symptoms in EDS.[9-11,13,15,20-24,26,41-44] In addition, muscle hypotonia, muscle rupture, fatigue, and musculoskeletal pain are included in the diagnostic criteria of EDS.[1] It is therefore remarkable that neuromuscular function in EDS has not been systematically studied before.

The discrepancy between moderate neuromuscular symptoms and only mild structural abnormalities on muscle biopsy may be related to non-neuromuscular problems in EDS: musculoskeletal pain, increased fatigability, and mild impairment may be due to articular and skeletal problems in EDS. Nevertheless, presence of myopathy and polyneuropathy points to an additional alternative explanation: abnormal composition of muscle ECM may play a role in the pathophysiology of muscular features.[7,11] Similarly, an altered peripheral nerve ECM may play a role in the pathophysiology of polyneuropathy in EDS.[16]

The findings of this study indeed support the hypothesis of a pathophysiological role of muscle and peripheral nerve ECM in neuromuscular features in EDS. First, the qualitative variation in the neuromuscular phenotype among the various EDS types may reflect the tissue specific distribution of the involved molecules. Collagen III (vascular type) and TNX (TNX-deficient type and hypermobility type with *TNXB* haploinsufficiency) are widely distributed in the ECM of both peripheral nerve and muscle, whereas collagen V (classical type) is only a minor component of peripheral nerve ECM.[45,46] This may explain peripheral nerve and muscle involvement in the vascular and TNX-deficient types, but absence of peripheral nerve dysfunction in the classical type of EDS.[45] Second, the inverse relation between residual TNX levels and degree of neuromuscular involvement may indicate a dose-effect relation both in muscle ECM (epimysium, perimysium, endomysium) and in peripheral nerve ECM (epineurium, perineurium, epineurium).[16] Third, reduction of density of collagen fibrils in the ECM of muscle on electron microscopy indicate that the composition

of the ECM is altered, which may indirectly support the pathophysiological role of the ECM in muscle function.

Abnormalities in muscle ECM composition may influence muscle function by altering myofascial force transmission.[7,11,47] This biomechanical theory proposes that in addition to myotendinous force transmission, force can be transmitted from muscle fibres onto the connective tissue within muscle and between adjacent muscle and compartments. This concept may help to understand why muscle strength is reduced in EDS, whereas muscle histology reveals only mild abnormalities, and CK is not elevated.[47]

Another possible explanation of muscle weakness due to ECM abnormalities may be related to mitochondrial dysfunction and increased apoptosis. This was recently detected in the collagen 6 myopathies Ullrich congenital muscular dystrophy and Bethlem myopathy. These two myopathies are also caused by ECM defects and show a remarkable overlap of dermal and articular features with EDS.[12,48-50] These findings illustrate how a genetic defect primary affecting the ECM influences cellular function.[51] Whether a similar pathophysiological mechanism is indeed involved in EDS needs to be investigated. Reduction of collagen VI mRNA expression in fibroblasts of *Tnxb* knockout mice may point in this direction.[52]

In conclusion, this study demonstrates neuromuscular features as part of EDS by showing mild to moderate neuromuscular involvement in four subtypes of EDS, with a dose-effect relation of residual TNX levels and degree of neuromuscular involvement. The results of this study further suggest that neuromuscular involvement in EDS is a direct effect of the ECM defect within muscle and peripheral nerve. This is in contrast with the widespread explanation of fatigue and musculoskeletal pain in EDS, i.e. that avoidance of exercise due to fear of dislocations and increased extensibility of tendons lead to muscle weakness and fatigue.[2]

Results of this study may contribute to increased recognition of neuromuscular features in various types of EDS, which is important for diagnosis and therapy. Results of this study also point to a biomechanical role of the ECM in muscle and peripheral nerve function, which has to be studied in further detail.[11,53]

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
3. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
4. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.
5. Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M, Kucharekova M, Boezeman JB, Schalkwijk J. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet 2003; 73: 214-217.
6. Zweers MC, Hakim AJ, Grahame R, Schalkwijk J. Joint hypermobility syndromes: the pathophysiologic role of tenascin-X gene defects. Arthritis Rheum 2004; 50: 2742-2749.
7. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
8. Pretorius ME, Butler IJ. Neurologic manifestations of Ehlers-Danlos syndrome. Neurology 1983; 33: 1087-1089.
9. Banerjee G, Agarwal RK, Shembesh NM, el Mauhoub M. Ehlers Danlos syndrome--masquerading as primary muscle disease. Postgrad Med J 1988; 64: 126-127.
10. Bilkey WJ, Baxter TL, Kottke FJ, Mundale MO. Muscle formation in Ehlers-Danlos syndrome. Arch Phys Med Rehabil 1981; 62: 444-448.
11. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
12. Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Ehlers-Danlos syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies. Am J Med Genet A 2007; 143: 2215-2219.
13. Schady W, Ochoa J. Ehlers-Danlos in association with tomaculous neuropathy. Neurology 1984; 34: 1270-1271.
14. Bell KM, Chalmers J. Recurrent common peroneal palsy in association with the Ehlers-Danlos syndrome. A case report. Acta Orthop Scand 1991; 62: 612-613.
15. Galan E, Kousseff BG. Peripheral neuropathy in Ehlers-Danlos syndrome. Pediatr Neurol 1995; 12: 242-245.
16. Voermans NC, Drost G, van Kampen A, Gabreels-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG. Recurrent neuropathy associated with Ehlers-Danlos syndrome. J Neurol 2006; 253: 670-671.
17. Beighton P, Price A, Lord J, Dickson E. Variants of the Ehlers-Danlos syndrome. Clinical, biochemical, haematological, and chromosomal features of 100 patients. Ann Rheum Dis 1969; 28: 228-245.
18. Kayed K, Kass B. Acute multiple brachial neuropathy and Ehlers-Danlos syndrome. Neurology 1979; 29: 1620-1621.
19. Curley SA, Osler T, Demarest GB. Traumatic disruption of the subclavian artery and brachial plexus in a patient with Ehlers-Danlos syndrome. Ann Emerg Med 1988; 17: 850-852.
20. Palmeri S, Mari F, Meloni I, Malandrini A, Ariani F, Villanova M, Pompilio A, Schwarze U, Byers PH, Renieri A. Neurological presentation of Ehlers-Danlos syndrome type IV in a family with parental mosaicism. Clin Genet 2003; 63: 510-515.
21. Muellbacher W, Finsterer J, Mamoli B, Bittner RE, Trautinger F. Axonal polyneuropathy in Ehlers-Danlos syndrome. Muscle Nerve 1998; 21: 972-974.
22. Farag TI, Schimke RN. Ehlers-Danlos syndrome: a new oculo-scoliotic type with associated polyneuropathy? Clin Genet 1989; 35: 121-124.
23. Chattopadhyay AK, Kandler RH, Sharrack B. The association of hereditary neuropathies and heritable skeletal disorders. Postgrad Med J 1995; 71: 245-246.
24. Bertin P, Treves R, Julia A, Gaillard S, sproges-Gotteron R. Ehlers-Danlos syndrome, clotting disorders and muscular dystrophy. Ann Rheum Dis 1989; 48: 953-956.

25. Giunta C, Randolph A, Al-Gazali LI, Brunner HG, Kraenzlin ME, Steinmann B. Nevo syndrome is allelic to the kyphoscoliotic type of the Ehlers-Danlos syndrome (EDS VIA). Am J Med Genet A 2005; 133: 158-164.
26. Yis U, Dirik E, Chambaz C, Steinmann B, Giunta C. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord 2008; 18: 210-214.
27. Forster HP, Emanuel E, Grady C. The 2000 revision of the Declaration of Helsinki: a step forward or more confusion? Lancet 2001; 358: 1449-1453.
28. Beighton P, Solomon L, Soskolne CL. Articular mobility in an African population. Ann Rheum Dis 1973; 32: 413-418.
29. Peterson-Kendall F, Kendall-McCreary E, Geise-Provance P, McIntyre-Rodgers M, Romani WA. Muscles testing and Function with Posture and Pain. Baltimore, MD, USA: Lipincott Williams & Wilkins; 2005.
30. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. J Neurol Neurosurg Psychiatry 1991; 54: 244-247.
31. Martina IS, van Koningsveld R, Schmitz PI, van der Meche FG, van Doorn PA. Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. European Inflammatory Neuropathy Cause and Treatment (INCAT) group. J Neurol Neurosurg Psychiatry 1998; 65: 743-747.
32. Thompson RA, Vignos PJ, Jr. Serum aldolase in muscle disease. AMA Arch Intern Med 1959; 103: 551-564.
33. Brooke MH, Griggs RC, Mendell JR, Fenichel GM, Shumate JB, Pellegrino RJ. Clinical trial in Duchenne dystrophy. I. The design of the protocol. Muscle Nerve 1981; 4: 186-197.
34. Collen FM, Wade DT, Robb GF, Bradshaw CM. The Rivermead Mobility Index: a further development of the Rivermead Motor Assessment. Int Disabil Stud 1991; 13: 50-54.
35. Rankin J. Cerebral vascular accidents in patients over the age of 60. III. Diagnosis and treatment. Scott Med J 1957; 2: 254-268.
36. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988; 19: 604-607.
37. England JD, Gronseth GS, Franklin G, Miller RG, Asbury AK, Carter GT, Cohen JA, Fisher MA, Howard JF, Kinsella LJ, Latov N, Lewis RA, Low PA, Sumner AJ. Distal symmetrical polyneuropathy: a definition for clinical research. A report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Arch Phys Med Rehabil 2005; 86: 167-174.
38. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. Muscle Nerve 2003; 27: 693-698.
39. Pillen S, Verrips A, van Alfen N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. Neuromuscul Disord 2007; 17: 509-516.
40. Arts IM, Pillen S, Overeem S, Schelhaas HJ, Zwarts MJ. Rise and fall of skeletal muscle size over the entire life span. J Am Geriatr Soc 2007; 55: 1150-1152.
41. Voermans NC, van Engelen BG. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord 2008; 18: 906.
42. Takaluoma K, Hyry M, Lantto J, Sormunen R, Bank RA, Kivirikko KI, Myllyharju J, Soininen R. Tissue-specific changes in the hydroxylysine content and cross-links of collagens and alterations in fibril morphology in lysyl hydroxylase 1 knock-out mice. J Biol Chem 2007; 282: 6588-6596.
43. Wenstrup RJ, Florer JB, Davidson JM, Phillips CL, Pfeiffer BJ, Menezes DW, Chervoneva I, Birk DE. Murine model of the Ehlers-Danlos syndrome. Col5a1 haploinsufficiency disrupts collagen fibril assembly at multiple stages. J Biol Chem 2006; 281: 12888-12895.
44. Schwarze U, Schievink WI, Petty E, Jaff MR, Babovic-Vuksanovic D, Cherry KJ, Pepin M, Byers PH. Haploinsufficiency for one COL3A1 allele of type III procollagen results in a phenotype similar to the vascular form of Ehlers-Danlos syndrome, Ehlers-Danlos syndrome type IV. Am J Hum Genet 2001; 69: 989-1001.
45. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. J Pathol 2003; 200: 423-428.
46. Matsumoto K, Sawa H, Sato M, Orba Y, Nagashima K, Ariga H. Distribution of extracellular matrix tenascin-X in sciatic nerves. Acta Neuropathol (Berl) 2002; 104: 448-454.

47. Huijing PA. Epimuscular myofascial force transmission between antagonistic and synergistic muscles can explain movement limitation in spastic paresis. J Electromyogr Kinesiol 2007.
48. Jobsis GJ, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA. Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. Nat Genet 1996; 14: 113-115.
49. Camacho VO, Bertini E, Zhang RZ, Petrini S, Minosse C, Sabatelli P, Giusti B, Chu ML, Pepe G. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. Proc Natl Acad Sci U S A 2001; 98: 7516-7521.
50. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, Anton-Lamprecht I, Muntoni F, Hanefeld F, Bonnemann CG. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A 2005; 132: 296-301.
51. Merlini L, Angelin A, Tiepolo T, Braghetta P, Sabatelli P, Zamparelli A, Ferlini A, Maraldi NM, Bonaldo P, Bernardi P. Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies. Proc Natl Acad Sci U S A 2008; 105: 5225-5229.
52. Minamitani T, Ariga H, Matsumoto K. Deficiency of tenascin-X causes a decrease in the level of expression of type VI collagen. Exp Cell Res 2004; 297: 49-60.
53. Previtali SC, Malaguti MC, Riva N, Scarlato M, Dacci P, Dina G, Triolo D, Porrello E, Lorenzetti I, Fazio R, Comi G, Bolino A, Quattrini A. The extracellular matrix affects axonal regeneration in peripheral neuropathies. Neurology 2008; 71: 322-331.

CHAPTER 5

Fatigue is a frequent and clinically relevant problem in Ehlers-Danlos Syndrome

Adapted from:
Voermans NC, Knoop J, van de Kamp N, Hamel BC, Bleijenberg G, van Engelen BG. Semin Arthritis Rheum. 2010;40:267-74.

Abstract

Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Fatigue and musculoskeletal pain are associated features, but have never been studied systematically.

We used a multidimensional assessment method to measure fatigue, its clinical relevance, and possible determinants. A questionnaire study was performed among 273 EDS patients. The following dimensions were assessed: fatigue severity, functional impairment in daily life, physical activity, psychological distress, sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, causal attribution of fatigue, pain, and disease related factors.

More than three quarter of EDS patients suffer from severe fatigue. Patients who are severely fatigued are more impaired than non-severely fatigued patients and report a higher level of psychological distress. The five possible determinants involved in fatigue are sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain severity.

This is the first study on fatigue and its possible determinants in EDS and shows that fatigue is a frequent and clinically significant problem in EDS. The five possible determinants of fatigue could form a starting point for the development of an effective cognitive behavioural intervention for fatigue in EDS.

Introduction

The Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders caused by mutations in genes encoding various types of collagen or collagen modifying enzymes.[1-3] The hypermobility type is the most common type of EDS, followed by the classical type.[4] Diagnostic criteria include joint hypermobility, skin hyperextensibility, and tissue fragility resulting in easy bruising and abnormal scarring.[1] Chronic joint/limb pain is one of the minor diagnostic criteria in the hypermobility type, and fatigue is acknowledged as an associated feature in the classic type.[1] Both symptoms generally receive little medical attention, although patients' reports suggest that these symptoms contribute significantly to the burden of disease in EDS.[5]

Fatigue can be defined as an overwhelming sense of tiredness, lack of energy, and feeling of exhaustion, and is not the same as muscle weakness.[6,7] Fatigue is a common symptom in various chronic diseases, and was found to be a major determinant of disability which significantly influences the quality of life.[7-12] The frequency of fatigue in EDS, its clinical relevance, and its associations with possible determinants have not yet been investigated together. We therefore performed an extensive questionnaire study on fatigue in EDS and used a multidimensional assessment method to measure fatigue, its clinical relevance and possible determinants in EDS. Questionnaire studies, due to their relatively unbiased and potential broad-ranged character, may shed light on the patients' perspective of fatigue and pain in EDS. This method has been used in several other chronic disorders and may identify possible determinants of fatigue in EDS, which can serve as a starting point for effective behavioural interventions.[6,13]

Methods

Patients

A cross sectional design was used to assess fatigue in patients with EDS. We asked 500 members of the Dutch patient organization of EDS (VED: www.ved.nl) to participate, and 19 additional patients were recruited from the outpatient departments of the Radboud University Nijmegen Medical Centre; these latter patients were also included in a clinical study on neuromuscular features in EDS.[14] The intention of the study was described as 'to learn more about various complaints in Ehlers-Danlos syndrome' to prevent a selection bias for fatigued patients. Written information on this purpose was provided to all patients, and all patients gave informed consent. 327 Questionnaires were returned (63% response rate). The study was approved by the local ethics committee.

Assessment

All patients were sent a booklet with: 1) various questions on EDS type; clinical features of EDS;[1] presence of muscle symptoms;[14] previous surgery and comorbidity; use of medication (based on a questionnaire assessing volumes of medical consumption in the previous six months);[15] three most important complaints in order of priority; and 2) seven validated questionnaires (see below).

Fatigue severity

The Checklist Individual Strength (CIS) measures four aspects of fatigue. We used the subscales fatigue severity (eight items, score range from 8 to 56) and concentration problems (five items, score range from 5 to 35).[6] High scores indicate more severe problems in the domain that is measured. A CIS-fatigue score of 35 or more was used to identify severe fatigue; this score is higher than the mean plus two standard deviations of a healthy control group.[6,16]

Dimensions of Fatigue

The multidimensional assessment method measures the following dimensions of fatigue: functional impairment in daily life, level of physical activity; psychological distress; sleep disturbances; concentration problems; social functioning and social support; self-efficacy concerning fatigue; causal attribution of fatigue; pain; and disease related factors.

Functional impairment in daily life

The Sickness Impact Profile (SIP) is aimed at measuring changes of conduct in everyday activities due to sickness.[13,17-19] For functional impairment, we used the subscales household management, work, and recreation and pastimes. Higher scores on a subscale indicates more disabilities in this domain. Physical disabilities were measured with the physical functioning subscale of the ShortForm-36 (SF36).[20] Scores range from 0 (maximum physical limitations) to 100 (optimal physical functioning).[21] Additionally, we included the number of weekly worked hours (paid and unpaid) as measures of functional ability. Unpaid work was defined as study, household duties, and volunteer work.

Physical activity

The level of physical activity was measured with the subscales mobility and ambulation of the SIP.

Psychological distress

The Beck Depression Inventory (BDI-pc) was used to measure clinical depression, which was defined as a score of > 4 (on a scale of 0 to 21),[22] Psychological distress was measured with the sumscore of the subscales anxiety and depression of the Symptom Check List (SCL-90).[23] Higher scores indicate a higher level of psychological distress.

Sleep disturbances

Sleep disturbances were measured with the sleep/rest scale of the SIP (reflecting disturbance of the normal wake and sleep pattern) and the sleep subscale of the SCL (reflecting quality of nocturnal sleep). Higher scores indicate more sleep disturbances. To differentiate between daytime and nighttime sleeping problems, we split the SIP sleep and rest subscale in items focusing on daytime sleeping, and the item focusing on nighttime sleep disturbances. Furthermore, we asked for the duration of night time and daytime sleep (hrs), and the occurrence of good quality of nighttime sleep (%).

Concentration problems

Perceived concentration problems were measured with the concentration subscale of the CIS. Furthermore, we used the alertness behaviour subscale of the SIP. Higher scores indicate more concentration problems.

Social functioning

Social functioning and social support was measured with the social interaction subscale of the SIP and the social functioning subscale of SF36. Higher scores indicate more impairment.

Self efficacy concerning fatigue

Self efficacy, a sense of control over fatigue symptoms, was measured with the Self Efficacy Scale (SES). The SES consisted of seven questions that measured sense of control with respect to fatigue. The total score ranges from 7 to 28, and a higher score reflects a higher sense of control.[24]

Causal attribution of fatigue

Patients were asked for their causal attribution of fatigue with the following options: EDS itself, too many activities, not enough leisure time, sleep disturbances, personal reasons, worrying thoughts, depressive thoughts, and a positive family history of fatigue. Patients could choose one or more attributions.

Pain

The visual analogous scale (VAS) scores for the current and the most severe pain (subscale of the McGill Pain Questionnaire) were filled in.[25] Pain was further assessed by the following three questions of the McGill Questionnaire: whether pain occurred (yes or no); whether it occurs at least 12 hours each day (% yes); and whether analgesics are used (% yes). Furthermore, we asked whether myalgia was present (% yes).

Disease related factors

Disease related factors questioned were hypermobility, dislocations, skin hyperextensibility, and easy bruising.[1] We added questions on muscle hypotonia, myalgia, and muscle weakness since we recently showed considerable neuromuscular involvement in various types of EDS.[14]

Impact of fatigue and impact of pain

The pain subscale of the SF36 was used to assess the impact of pain; it measures the magnitude of pain and its resulting self-reported functional limitations due to pain. Scores range from 0 to 100 (0 = no pain and no impairments due to pain; 100 = maximal pain and maximal impairment due to pain). We used the same items and scores again, replacing the word pain by fatigue, to be able to compare the impact of fatigue (magnitude of fatigue and self-reported functional limitations due to fatigue) with the impact of pain.

Statistics

SPSS for Windows (version 16.0) was used to carry out data analysis, and we used descriptive statistics to depict the sample. We defined two groups of patients: severely fatigued patients (CIS fatigue ≥ 35) versus non-severely fatigued patients (CIS fatigue < 35).[6,16] We tested the differences in scores on the various measures between the non-severely and severely fatigued patients with a Chi-square or Fisher's exact test (dichotomous variables), Student's t-test (variables at least interval level), or a Mann-Whitney-U test (ordinal variables). Correlations were calculated with the Pearson coefficient (r). Probability (P) values smaller than 0.05 were regarded as statistically significant. For each dimension, we selected the measure with the strongest and statistical significant correlation with the CIS-fatigue score for subsequent multiple linear regression analysis, in which we determined the contribution of various measures to the level of fatigue (backward method; p in = 0.01; p out = 0.05). The measures that resulted from this regression analysis were considered to be possible determinants of fatigue.

Results

Excluded were patients who were younger than 16 years (n = 16), patients in whom EDS had not been diagnosed (n = 30), and patients who had filled out the CIS fatigue severity scale incompletely (n = 8). Hence, 273 patients were included; in 53 of them the specific type of EDS was not (yet) known although EDS was diagnosed by a medical specialist; in all other patients the EDS type was classified according to the diagnostic criteria.[1] Demographic characteristics of the patients are summarized in *Table 1*.

Three most important symptoms

264 patients completed the questionnaire on most important complaints. The mean number of self-reported complaints was 5.6 (SD 2.5), and 204 patients reported at least four complaints. Musculoskeletal pain, hypermobility/dislocations, and fatigue were most frequently reported spontaneously: 99% of the patients reported musculoskeletal pain as

Table 1 Demographics of Ehlers-Danlos syndrome (EDS) patients. Distinction between non-severely and severely fatigued patients.

EDS type	Number of patients	Mean age (range)	Female (% of type)	EDS Non-severely fatigued (% of type)	EDS Severely fatigued (% of type)	Stat.sign.[1] of difference (P)
Classical	45	43.1 (16-68)	35 (78)	14 (31)	31 (69)	0.032 clas - hyper
Hypermobility	162	38.8 (16-74)	152 (94)	26 (16)	136 (84)	
Vascular	11	33.6 (19-61)	9 (82)	5 (45)	6 (55)	
Kyphoscoliotic	2	21.0 (16-26)	0 (0)	2 (100)	0 (0)	
Other / Type unknown	53	46.2 (19-89)	48 (91)	15 (28)	38 (72)	
Total	273	40.7 (16-89)	244 (89)	62 (23)	211 (77)	

[1] Fisher's exact test. clas: classical type; hyper: hypermobility type.

one of their three most important complaints; 63% hypermobility/dislocations; and 57% fatigue. In contrast, dermal features (skin hyperextensibility, easy bruising, abnormal scar formation) were far less often reported, although they are part of the diagnostic criteria.[1]

Fatigue severity and dimensions

The mean CIS fatigue score was 41.7 (SD 11.3). The majority of patients (77%) was severely fatigued (CIS fatigue ≥ 35). Severe fatigue was more common in the hypermobility type than in the classic type (P = 0.032)(*Table 1*). Age, sex, and level of education did not differ between severely and non-severely fatigued patients. *Table 2* shows the differences between non-severely and severely fatigued patients on the various dimensions. In all dimensions, being severely fatigued was associated with more problems or limitations.

Previous surgery, comorbidity, and use of medication

Previous surgery of any kind was reported by 247 patients (90%). The patients who were operated before had a higher CIS fatigue score than those who were not (mean (SD): 42.4 (11.0) vs. 34.5 (11.9); P = 0.001). 117 (43%) Patients reported any kind of comorbidity, and this group had a higher mean CIS fatigue score than the patients without self-reported co-morbidity (mean (SD): 43.9 (9.3) vs. 40.0 (12.4); P = 0.003). Among the patients with comorbidity, anaemia was mentioned by three patients, and hypothyroidism by two. Analgesics with possible sedative (side-) effects were used by 62 patients, and these patients

Table 2 Differences between non-severely and severely fatigued patients on ten dimensions, each of which is assessed by one or more measures (left columns). Correlation of each of these measures with the CIS fatigue score is given in the right columns. The measure with the highest correlation coefficient of each dimension is highlighted. These measures were used in the multiple regression analysis (Table 4).

	EDS Non-severely fatigued *mean (SD) / %*	**EDS Severely fatigued** *mean (SD) / %*	**Sign. testing[1] P**	**Pearson correlation (unless otherwhise specified)** *Correlation coefficient r*	*Stat.sign. of correlation a*
1) Functional impairment in daily life					
SF36 physical functioning	61 (27)	37.1 (23)	< 0.001	-0.498	0.000
SIP work problems	96 (145)	198 (163)	< 0.001	0.343	0.000
SIP household management	119 (131)	195 (116)	< 0.001	0.341	0.000
SIP recreation and pastime	58 (63)	123 (76.4)	< 0.001	0.403	0.000
Weekly worked hours (paid)	14 (19)	5 (14)	0.001	- 0.332	0.000
Weekly worked hours (unpaid)	8 (17)	7 (19)	n.s.	- 0.022	n.s.
2) Physical activity					
SIP mobility	39 (68)	94 (114)	< 0.001	0.344	0.000
SIP ambulation	83 (102.0)	156 (120)	< 0.001	0.319	0.000
3) Psychological distress					
% BDI PC > 4	3	20	0.0102	0.337	0.000
SCL psychological distress	33 (7.3)	42 (16)	< 0.001	0.420	0.000
4) Sleep disturbances					
SIP sleep and rest Daytime sleep	30 (34)	68 (59)	< 0.001	0.409	0.000
SIP sleep and rest Nighttime sleep	11 (24)	31 (31)	< 0.001	0.338	0.000
SIP sleep and rest	42 (44)	100 (70)	< 0.001	0.478	0.000
SCL sleep disturbances	4 (2)	7 (3)	< 0.001	0.462	0.000
Duration nighttime sleep (hrs)	8 (1)	7 (2)	n.s.	- 0.078	n.s.
Duration daytime sleep (hrs)	0.3 (0.7)	0.9 (1)	< 0.001	0.264	0.000
% Good quality of nighttime sleep	61	32	< 0.001[2]	- 0.290[4]	0.000
5) Concentration problems					
CIS concentration	11 (7)	16 (9)	< 0.001	0.359	0.000
SIP alertness behaviour	62 (80)	153(158)	< 0.001	0.357	0.000

Table 2 Continued.

6) Social functioning and social support					
SIP social interaction	82 (116)	190 (180)	< 0.001	0.404	0.000
SF36 social functioning	80 (21)	54 (23)	< 0.001	- 0.557	0.000
7) Self-efficacy concerning fatigue					
SES28	21 (4)	18 (4)	< 0.001	- 0.403	0.000
8) Causal attribution of fatigue					
% Ehlers-Danlos syndrome	82	94	0.010[3]	0.186[4]	0.003
% Too many activities	27	13	0.012[2]	- 0.155[4]	0.012
% Not enough leisure time	6	6	n.s.[3]	- 0.059[4]	n.s.
% Sleep disturbances	19	37	0.013[2]	0.232[4]	0.000
% Personal reasons	2	7	n.s.[3]	0.001[4]	n.s
% Worrying thoughts	17	19	n.s[2]	0.059[4]	n.s.
% Depressive thoughts	6	10	n.s.[3]	0.119[4]	n.s.
% Family history of fatigue	6	4	n.s.[3]	- 0.030	n.s.
9) Pain					
% Occurrence of pain	69	96	0.001[2]	0.387[4]	0.000
% Daily pain duration > 12 hours	20	54	0.001[2]	0.210[4]	0.002
% Use of analgesics	56	86	0.001[2]	0.180[4]	0.000
% Occurrence of frequent or continuous myalgia	65	87	< 0.001	0.380[4]	0.000
Current pain: VAS	4 (3)	5 (2)	0.002	0.373	0.000
Most severe pain: VAS	5 (4)	8 (2)	0.001	0.540	0.000
10) Disease related factors					
% Hypermobility	91	95	n.s.[3]	0.104[4]	n.s.
% Dislocations	76	78	n.s.[2]	0.049[4]	n.s.
% Skin hyperextensibility	57	48	n.s.[2]	- 0.080[4]	n.s.
% Easy bruising	89	83	n.s.[2]	- 0.064[4]	n.s.
% Muscle weakness	61	80	0.0039[2]	0.167[4]	0.006
% Muscle hypotonia	43	56	n.s.[2]	0.209[4]	0.001

[1] Independent t-test unless otherwise specified. [2] Chi-Square test. [3] Fisher's Exact test. [4] Spearmans's rho; non-parametric correlation. SD = standard deviation.

had a higher CIS fatigue than those not using these medications (46.7 (SD 7.9) vs. 40.1 (SD 11.8); P < 0.001). Antidepressive drugs with possible sedative (side-)effects were used by 38 patients, and the CIS fatigue did not differ significantly in both groups (44.1 (SD 10.5) vs. 41.7 (SD 11.0); P = 0.24). Use of benzodiazepines was reported by 39 patients, and these patients had a higher CIS fatigue than those not using (46.5 (SD 9.4) vs. 40.8 (SD 11.4); P = 0.003). Use of

beta-blockers was reported by 6 patients, and the CIS fatigue did not differ from the patients not using them (39.2 (SD 6.6) vs. 41.8 (SD 11.4); P = 0.59). *Table 3* summarizes the (categories of) diagnoses reported as co-morbidity and the self-reported medication with possible sedative (side-)effects.

Table 3 Self-reported co-morbidity and use of medications with possible sedative (side-)effects.

Self-reported (category) of co-morbidity	Number of EDS patients
Anaemia	3
Hypothyroidism	2
Hypertension	11
Diabetes mellitus	2
Other internal diseases	21
Osteoarthritis	5
Other reumatological diseases	25
Chronic obstructive pulmonary disease / Bronchial asthma	22
Migraine / other headaches	4
Other neurological diseases	5
Depression	2
Burnout	3
Other psychiatric diseases	8
Allergy	14
Dermatological diseases	6
Gynaecological diseases	5
Ophthalmological diseases	4
Cardiac disease	6
Self-reported use of medication with possible sedative (side-)effects	
One or more analgesics with sedative (side-) effects	62
Tramadol	14
Opiates / other opiate agonists	39
Codeine	22
Antiepileptic drugs	3
Baclofen	2
One or more antidepressive drugs with possible sedative (side-)effects	38
One or more benzodiazepines	39
One or more beta-blockers	6

Regression analysis

The measures with the highest correlation coefficient for each dimensions were selected to be included in the multiple regression analysis; these measures are highlighted in *Table 2*. Regression analysis with CIS fatigue as dependent variable resulted in a model with five possible determinants: disturbances in sleep and wake pattern (SIP sleep and rest), concentration problems (CIS concentration), social functioning (SF36 social functioning), self efficacy concerning fatigue (SES28), and pain (most severe pain: VAS). Together, these possible determinants predicted 38% of the fatigue severity (*Table 4*).

Table 4 Multiple regression analysis of possible determinants of fatigue. Results of multiple linear regression analysis, in which we determined the contribution of one measure of each dimension to the level of fatigue. This analysis resulted in a model with five possible determinants, which are highlighted in the table below. This model predicted 38% of the fatigue severity score of the CIS.

Independent variables		**Dependent variable CIS fatigue**	
Dimension	*Possible determinant*	*beta*	*Stat.sign. P*
1) Functional impairment in daily life	SF36 physical functioning		
2) Physical activity	SIP mobility		
3) Psychological distress	SCL negative affectivity		
4) Sleep disturbances	SIP sleep and rest	0.136	0.028
5) Concentration problems	CIS concentration	0.148	0.007
6) Social functioning and social support	SF36 social functioning	- 0.229	< 0.001
7) Self efficacy concerning fatigue	SES28	- 0.240	< 0.001
8) Causal attribution of fatigue	Sleep disturbances		
9) Pain	Most severe pain: VAS	0.220	< 0.001
10) Disease related factors	Muscle hypotonia		

Backward; p in = 0.01; p out = 0.05; Adjusted R2 = 0.382.

Impact of fatigue and impact of pain

Pain severity (most severe pain (VAS)) was higher in the severely fatigued than in non-severely fatigued patients (P = 0.001). In contrast, fatigue had more frequently a larger impact on daily functioning than pain (P < 0.001): in 40% of the EDS patients fatigue had a larger impact than pain (SF36 fatigue > SF36 pain); for 34% of the patients the impact of pain was equivalent to that due to fatigue (SF36 pain = SF36 fatigue); and 26% reported a larger impact of pain symptoms than of fatigue (SF36 fatigue < SF36 pain).

Discussion

This is the first in-depth questionnaire study on fatigue in EDS, showing that fatigue is a frequent and clinically relevant problem in EDS: 77% of EDS patients suffer from severe fatigue; and patients who are severely fatigued are more impaired and report a higher level of psychological distress. Patients with the hypermobility type EDS are most often severely fatigued. Furthermore, severe fatigue in EDS is related to sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain.

The prevalence of 77% severe fatigue and the mean CIS fatigue score of 41.7 is high in comparison with the general population. In a Dutch study on chronic fatigue in disease-free breast cancer patients, a control group of 78 healthy females (mean age 48.1 (SD 6.2) years) was used. The mean CIS fatigue in this group was 19.4 (SD 11.0), and 11% of the women had a CIS fatigue of 35 or more.[12] Another study reported a mean CIS fatigue of 17.3 (SD 10.1) in a group of 53 healthy Dutch controls with predominantly female subjects.[26] The prevalence of severe fatigue in this EDS population was also high in comparison with rheumatoid arthritis, another chronic disorder with extensive articular involvement and high prevalence of pain. A recent study among 150 rheumatoid arthritis patients reported a mean CIS fatigue of 34.2 (SD 10.2), with 52% of the patients being severely fatigued.[11]

Many EDS patients in this study reported previous surgery and one or more disorder as comorbidity. Patients with previous surgery or comorbidity had a higher mean CIS fatigue score than those without, although in both groups this was above 35. However, anemia and hypothyroidism, disorders known to cause fatigue, were very rare. Furthermore, the prevalence of medication use with possible sedative (side-)effects is high in this population. The mean CIS fatigue score in the patients using analgesics with possible sedative side effects or benzodiazepines was higher than in the other EDS patients. Most likely, use of this medication contributes to the high prevalence of severe fatigue in this population. The limitation of these data is that it is based on self-reports, and this may not be very accurate. Furthermore, it is difficult to evaluate the individual contribution of these factors to fatigue, since they are probably interrelated, and dependent on the severity of EDS.

We found that pain is another common and clinically relevant symptom in EDS, which is in line with the results of a previous study on pain in EDS.[27] In fact, pain was more often spontaneously reported than fatigue, whereas fatigue had a larger impact than pain on daily functioning. Hence, our study shows that both pain and fatigue contribute to the burden of disease in EDS. Therefore, fatigue and pain should preferably be evaluated collectively and addressed simultaneously in symptomatic treatment. Pain and fatigue are mentioned as associated features in the diagnostic criteria,[1] but generally receive little attention in medical practice and literature. The finding that these symptoms are very often spontaneously reported as most important complaint, that they are severe, and

have a large impact emphasize the importance of questionnaire studies.

We identified five possible determinants of fatigue in EDS: sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain. Since we used cross sectional data, we were only able to show that these factors are possible determinants. However, a strong association of these possible determinants with severe fatigue is suggested by this study. More precisely, sleep disturbances,[28] low self-efficacy concerning fatigue, and pain most likely contribute to persistence of severe fatigue. Sleep problems in EDS were previously found to consist predominantly of difficulties initiating and maintaining sleep.[29] We added the finding that a significant number of patient rest and sleep during the day, which was associated with fatigue severity. Concentration problems may rather be a result of fatigue, whereas social impairment might be either a perpetuating factor or a consequence of fatigue.[12,30] A follow-up study would allow to analyze whether these possible determinants are cause or consequence of fatigue.

A limitation of this study is the ascertainment bias introduced by addressing members of a patients support group, and only using the questionnaires which were returned. Both patients who are not severely impacted by the disease and busy with their work and family and patients who are severely affected with major impairment might not get involved in a patient support group and tend not to complete questionnaires. However, recruitment of consecutive EDS patients at an outpatients department is complicated, since the care for EDS patients is multidisciplinary, and most hospitals do not have a specialized EDS centre. Furthermore, we were unable to perform a non-responders analysis, since the patient support group took care of the mailing of the questionnaires and did not send us the names and addresses of their members for privacy reasons.

The results of our study can serve as a starting point for symptomatic treatment approaches. A cognitive behavioural intervention focusing on pain, sleep disturbances, the reaction of others to the symptoms and self efficacy concerning fatigue could help to reduce fatigue and fatigue related disabilities. Positive results of this approach can be expected from recent findings in other chronic diseases with high frequency of severe fatigue.[31-33] Furthermore, medical interventions should address pain and evaluate medication use, and irregular sleeping patterns should be analyzed for treatable causes.

In short, this is the first in-depth questionnaire study on fatigue in EDS, showing that fatigue is a frequent and clinically relevant problem in EDS.

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
3. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.
4. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
5. Gawthrop F, Mould R, Sperritt A, Neale F. Ehlers-Danlos syndrome. BMJ 2007; 335: 448-450.
6. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. J Psychosom Res 1994; 38: 383-392.
7. Kalkman JS, Schillings ML, van der Werf SP, Padberg GW, Zwarts MJ, van Engelen BG, Bleijenberg G. Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and HMSN-I. J Neurol Neurosurg Psychiatry 2005; 76: 1406-1409.
8. Schwid SR, Covington M, Segal BM, Goodman AD. Fatigue in multiple sclerosis: current understanding and future directions. J Rehabil Res Dev 2002; 39: 211-224.
9. Friedman J, Friedman H. Fatigue in Parkinson's disease. Neurology 1993; 43: 2016-2018.
10. van der Werf SP, van den Broek HL, Anten HW, Bleijenberg G. Experience of severe fatigue long after stroke and its relation to depressive symptoms and disease characteristics. Eur Neurol 2001; 45: 28-33.
11. Repping-Wuts H, Fransen J, van Achterberg T, Bleijenberg G, van Riel P. Persistent severe fatigue in patients with rheumatoid arthritis. J Clin Nurs 2007; 16: 377-383.
12. Servaes P, Verhagen CA, Bleijenberg G. Relations between fatigue, neuropsychological functioning, and physical activity after treatment for breast carcinoma: daily self-report and objective behavior. Cancer 2002; 95: 2017-2026.
13. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. The development of a model of fatigue in neuromuscular disorders: a longitudinal study. J Psychosom Res 2007; 62: 571-579.
14. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
15. Scheeres K, Wensing M, Bleijenberg G, Severens JL. Implementing cognitive behavior therapy for chronic fatigue syndrome in mental health care: a costs and outcomes analysis. BMC Health Serv Res 2008; 8: 175.
16. Vercoulen JH, Hommes OR, Swanink CM, Jongen PJ, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. The measurement of fatigue in patients with multiple sclerosis. A multidimensional comparison with patients with chronic fatigue syndrome and healthy subjects. Arch Neurol 1996; 53: 642-649.
17. Berger F, Brahler E, Kunkel R, Stephanos S. [Verbal behavior and communication experience of psychosomatic patients in the first psychoanalytic interview in connection with the concept of "pensee operatoire']. Z Psychosom Med Psychoanal 1981; 27: 45-58.
18. Jacobs HM, Luttik A, Touw-Otten FW, de Melker RA. [The sickness impact profile; results of an evaluation study of the Dutch version]. Ned Tijdschr Geneeskd 1990; 134: 1950-1954.
19. Pfeiffer G, Wicklein EM, Ratusinski T, Schmitt L, Kunze K. Disability and quality of life in Charcot-Marie-Tooth disease type 1. J Neurol Neurosurg Psychiatry 2001; 70: 548-550.
20. Ware JE, Snow KK, Kosinski M. SF-36 Health Survey Manual and Interpretation Guide. Boston, MA: New England Medical Center, The Health Institute. 2008.
21. Stewart JW, Quitkin FM, McGrath PJ, Rabkin JG, Markowitz JS, Tricamo E, Klein DF. Social functioning in chronic depression: effect of 6 weeks of antidepressant treatment. Psychiatry Res 1988; 25: 213-222.
22. Beck AT, Guth D, Steer RA, Ball R. Screening for major depression disorders in medical inpatients with the Beck Depression Inventory for Primary Care. Behav Res Ther 1997; 35: 785-791.
23. Arrindell WA, Ettema JHM. SCL-90-R: Handleiding bij een multidimensionele psychopathologie-indicator. Lisse: Swets & Zeitlinger; 1986.

24. Prins JB, Bleijenberg G, Bazelmans E, Elving LD, de Boo TM, Severens JL, van der Wilt GJ, Spinhoven P, van der Meer JW. Cognitive behaviour therapy for chronic fatigue syndrome: a multicentre randomised controlled trial. Lancet 2001; 357: 841-847.
25. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1975; 1: 277-299.
26. Vercoulen JH, Alberts M, Bleijenberg G. De Checklist Individual Strength (CIS). 1999. p. 131-6.
27. Sacheti A, Szemere J, Bernstein B, Tafas T, Schechter N, Tsipouras P. Chronic pain is a manifestation of the Ehlers-Danlos syndrome. J Pain Symptom Manage 1997; 14: 88-93.
28. Naughton F, Ashworth P, Skevington SM. Does sleep quality predict pain-related disability in chronic pain patients? The mediating roles of depression and pain severity. Pain 2007; 127: 243-252.
29. Verbraecken J, Declerck A, van de Heyning P, de Backer W, Wouters EF. Evaluation for sleep apnea in patients with Ehlers-Danlos syndrome and Marfan: a questionnaire study. Clin Genet 2001; 60: 360-365.
30. Prins JB, Bos E, Huibers MJ, Servaes P, van der Werf SP, van der Meer JW, Bleijenberg G. Social support and the persistence of complaints in chronic fatigue syndrome. Psychother Psychosom 2004; 73: 174-182.
31. Prins JB, van der Meer JW, Bleijenberg G. Chronic fatigue syndrome. Lancet 2006; 367: 346-355.
32. Gielissen MF, Verhagen S, Witjes F, Bleijenberg G. Effects of cognitive behavior therapy in severely fatigued disease-free cancer patients compared with patients waiting for cognitive behavior therapy: a randomized controlled trial. J Clin Oncol 2006; 24: 4882-4887.
33. Milne HM, Wallman KE, Gordon S, Courneya KS. Effects of a combined aerobic and resistance exercise program in breast cancer survivors: a randomized controlled trial. Breast Cancer Res Treat 2008; 108: 279-288.

CHAPTER 6

Pain in Ehlers-Danlos syndrome is common, severe, and associated with fatigue and functional impairment

Adapted from:
Voermans NC, Knoop J. Bleijenberg G, van Engelen BG.
J Pain Symptom Manage. 2010;40:370-8.

Abstract

The Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Musculoskeletal pain is mentioned in the diagnostic criteria and described as early in onset, chronic, and debilitating. However, systematic research on pain in EDS is scarce. Therefore, we investigated prevalence and impact of pain and associated features in a large group of EDS patients.

We performed a study among members of the Dutch EDS patient organization (n = 273), and included the McGill Pain Questionnaire to investigate various aspects of pain, the Sickness Impact Profile to study functional impairment, the Symptom Check List subscale sleep to evaluate sleep disturbances, and the Checklist Individual Strength subscale fatigue to determine fatigue severity.

The results of this study show that 1) chronic pain in EDS is highly prevalent and associated with regular use of analgesics; 2) pain is more prevalent and more severe in the hypermobility type than in the classical type; 3) pain severity is correlated with hypermobility, dislocations, and previous surgery; 4) pain is correlated with low nocturnal sleep quality; and 5) pain contributes to functional impairment in daily life, independently of the level of fatigue.

From this large cohort of EDS patients, we conclude that pain is common and severe in EDS. Pain is related to hypermobility, dislocations, and previous surgery and associated with moderate to severe impairment in daily functioning. Therefore, treatment of pain should be a prominent aspect of the symptomatic management of EDS.

Introduction

The Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders, caused by mutations in genes encoding various types of collagen or collagen modifying enzymes (collagen I, III, V, tenascin-X (TNX) or lysyl hydroxylase-1). The collagen in connective tissue increases its elasticity and thus helps tissues to resist deformation. In the skin, muscles, ligaments, blood vessels, and visceral organs collagen plays a very significant role. Reduced elasticity secondary to abnormal collagen in EDS results in joint hypermobility, skin hyperextensibility, tissue fragility, and possibly to ruptures of visceral organs and blood vessels.

The revised classification of EDS in six major types is based upon clinical and biochemical features.[1] The hypermobility type is the most common type, followed by the classical type; together these types account for 90% of cases.[2] The vascular, kyphoscoliotic, arthrochalasia, and dermatosparaxis types are rare, as is the TNX-deficient type which has been described more recently.[3,4] Musculoskeletal pain is mentioned in the diagnostic criteria of the hypermobility type and described as early in onset, chronic, and possibly debilitating.[1,5] This latter aspect is clearly illustrated by a recent patients' report of pain in EDS.[6]

Systematic research on pain in EDS is restricted to a questionnaire study on 51 patients, which showed that moderate to severe pain is common in EDS. Pain was often chronic and multifocal and suggested to have several causes; secondary to frequent dislocations, resulting from repeated soft tissue injury, or related to multiple operations with peripheral nerve injury.[5] This multifactorial basis was assumed to cause a variable course of pain in EDS: pain related to repeated soft tissue injury or multiple operations with peripheral nerve injury was thought to cause a constant level of pain, whereas hypermobility and dislocations may lead to additional peaks of pain.[5] Differences between EDS types could not be tested due to the small sample size.[5] Other studies on general symptoms in EDS also reported high prevalence of pain, but did not focus on specific pain characteristics.[7-10]

Pain was found to be a prominent symptom in our questionnaire study on fatigue in EDS (90%),[11] and myalgia was frequently reported in our study on neuromuscular features in EDS (73%).[12] Furthermore, the previous study on pain in EDS was restricted in size and focus.[5] Therefore, we investigated prevalence, characteristics, and impact of pain in a large group of EDS patients. Our aims were: 1) to determine pain prevalence, pain characteristics, and use of analgesics (to replicate the findings of previous studies); 2) to investigate whether differences in pain prevalence and impact of pain occur between the two most common types of EDS (hypermobility and classic type); 3) to explore the relation between pain and disease-related factors. Since sleep disturbances are common in EDS,[13] we also wanted to 4) investigate the relation of pain with sleep disturbances. Finally, we meant to 5) assess the contribution of pain to functional impairment in daily life. Since previous research has shown that pain and

fatigue are associated,[14] we controlled for the contribution of fatigue when determining the relationship between pain and level of disabilities. This study may thus contribute to a better recognition and understanding of pain in EDS, which is a necessary starting point for treatment protocols for pain in EDS.

Methods

Patients

We used a cross sectional design to assess fatigue and pain in patients with EDS. In total, 519 patients were asked to participate (500 questionnaires were sent to members of the Dutch patient organization of EDS (VED: www.ved.nl), and 19 patients were recruited from the outpatient departments of internal medicine, dermatology, and human genetics of the Radboud University Nijmegen Medical Centre). These 19 patients were also included in our clinical study on neuromuscular features in EDS.[12] Three hundred twenty-seven questionnaires were returned (63% response rate). Questionnaires were provided on paper, patients were asked to fill them out by themselves, and return them by mail. We excluded patients who were younger than 16 years, patients in whom EDS had not (yet) been diagnosed, and patients who had filled out the CIS questionnaire incompletely.

Written information about the purpose of the study was provided, and all patients gave informed consent. To prevent a selection bias for patients the purpose of the study was described as 'to learn more about various complaints in Ehlers-Danlos syndrome'. The study was approved by the local ethics committee.

Assessment of pain

Prevalence of pain, pain characteristics, and use of analgesics

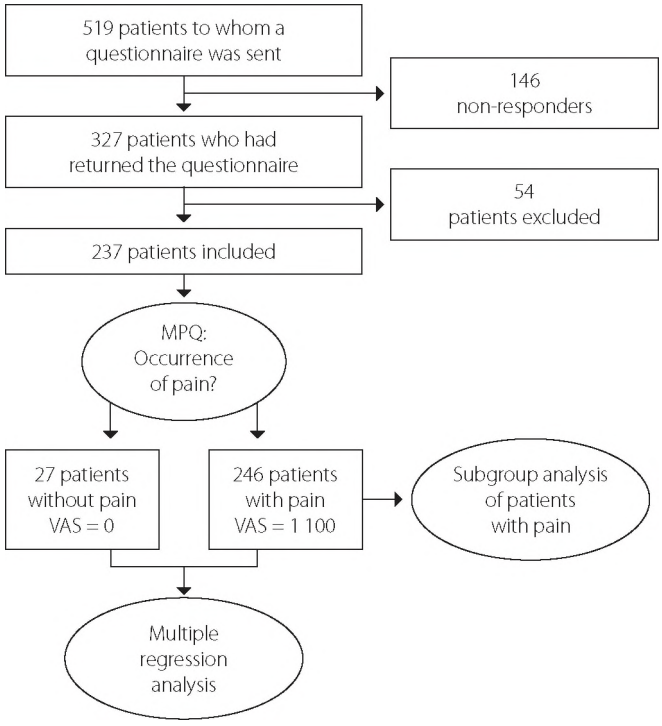
We used the McGill Pain Questionnaire because it provides qualitative and quantitative data on various aspects of pain in a standardized way.[15] The first question concerns the occurrence of pain. If no pain is reported, the subsequent questions on this questionnaire do not have to be filled out, and patients are referred to the next questionnaire in the booklet. Therefore, only the patients that reported having pain were included for the subsequent subgroup analyses of pain severity, pain characteristics, use of analgesics, differences between two EDS types, relation of pain with disease-related factors and sleep disturbances, and impact of pain (See: Flow chart in *Figure 1*). For the multiple regression analysis, we used all 273 patients (See: Flow chart in *Figure 1* and paragraph on Statistics below).

The subsequent questions of the McGill pain Questionnaire focus on pain severity, changes of pain over time, and specific characteristics of the pain. The pain severity is scored as current pain, most severe pain, and least severe pain, measured with the visual analogous

scale (VAS): a score of 0 indicates no pain, and a 100 indicates unbearable pain. Pain at the day of filling out the questionnaire is scored as none – mild – moderate – severe. Pain characteristics are assessed with use of a list of adjectives reflecting pain quality and intensity, of which patients have to select the most appropriate ones. Use of analgesics is qualitatively assessed with the McGill questionnaire. Additionally, we used the items on a health care use questionnaire that assessed doses of (non)prescribed medication to screen whether patients exceeded the maximally allowed doses.[16]

In our study on neuromuscular features in EDS, muscle weakness and myalgia were frequently reported by EDS patients (65% and 73% respectively).[12] Therefore, we also asked a question on myalgia, how often this occurred, and which parts of the body were involved.

Figure 1 Flow chart of the inclusion and exclusion of EDS patients in this study.



The relation of pain with disease-related factors

We asked patients about the previous or current presence (yes or no) of joint hypermobility, dislocations, dermal features (velvety skin; hyperextensible skin; easy bruising; presence of varices; thin, translucent skin; molluscoid pseudotumors; impaired wound healing; scars that are thin, wide and discoloured and / or have an atrophic appearance; and presence of striae), muscle weakness, and about previously performed operations (with explanations of medical terms).

The relation of pain with sleep disturbances

The McGill questionnaire includes items on waking up during the night due to pain, and on the duration of being awake at night. Sleeping problems were further measured with the sleep subscale of the Symptom Check List (SCL-90). This scale consists of three items that focus on the quality of sleep (difficulty falling asleep, early awakening, and sleep disturbances during the night) which must be rated on a 5-point scale.[17] A total score is obtained by adding the item scores, scores can range from 3 (not bothered by sleep problems) to 15 (high level of distress because of sleep problems).

Contribution of pain and fatigue to functional impairment in daily life

We used the McGill Pain questionnaire items on functional limitations due to pain. These functional consequences of the pain are scored in the following order: none – mild – moderate – severe, and concern limitations in daily activities, both work and leisure activities, appetite, and lack of energy. Physical disabilities were measured with the physical functioning subscale of the ShortForm-36 (SF36).[18] Scores range from 0 (maximum physical limitations) to 100 (optimal physical functioning).[19] Additionally, the Sickness Impact Profile (SIP) was used to assess functional disability. The SIP is a standardized list of statements aimed at measuring changes of conduct in everyday activities due to sickness. We used eight SIP categories: sleep and rest, home management, ambulation, social interaction, mobility, alertness behaviour, work, and recreation and pastimes. Higher scores mean more functional disability.[20-23] We used the SIP sum score to assess the level of overall disabilities.[19]

The Checklist Individual Strength (CIS) subscale fatigue severity consists of eight items. Each item was scored on a seven point Likert scale (scores range from 8 to 56). High scores indicated high levels of fatigue. The CIS has good reliability and validity.[24]

Statistics

Data analysis was carried out using SPSS for Windows (version 16.0). Descriptive statistics were used to describe the sample. For the analyses of pain characteristics and impact of pain, only patients that reported having pain on the first question of McGill pain questionnaire were included. Differences in prevalence and severity of pain between the classical and hypermobility types were tested with the independent t-test for continuous variables and

with a Chi-square or Fisher's exact test for dichotomous variables. Correlations were calculated with the Pearson coefficient or Spearman's coefficient. Multiple regression analysis was performed (enter method; p in = 0.05; p out = 0.1); with functional impairment (SIP sum score) as dependent variable. For this analysis, we used the data of all patients, and designated 'no pain' as a VAS score of 0 (See Flow chart in *Figure 1*). Pain severity (VAS of most severe pain; VAS of current pain) and fatigue (CIS fatigue) were defined as predictors. In all analyses probability (P) values smaller than 0.05 were regarded as statistically significant.

Results

Patients

In total, 327 questionnaires were returned. Excluded were patients who were younger than 16 years (n = 16), patients in whom EDS had not (yet) been diagnosed (n = 30), and patients who had incompletely filled out the CIS questionnaire (n = 8). Hence, 273 patients were included. The mean age of EDS patients was 41 years (range 16 - 89), and 89% of the patients was female. Highest level of education was in 16% of patients low (primary school, low vocational school); middle in 47% (high school, middle vocational school), and high (higher vocational school, university) in 37%. EDS was diagnosed by a medical specialist in all patients. In 53 of them (91% female), the specific type of EDS was not (yet) known; in all other patients the EDS type was diagnosed based upon clinical features, and partly supported by biochemical or genetic analysis: 45 EDS patients of the classical type (78% female); 162 of the hypermobility type (94% female), 11 of the vascular type (82% female), and two of the kyphoscoliotic type (0% female).[1] No differences were found in age or gender between the various EDS types or between patients with or without classification. Overall, 237 patients (87%) had undergone surgery at least once; this included various orthopaedic operations (all types) and vascular surgery after ruptured aneurysm or abdominal surgery after ruptured intestines (vascular type).

Assessment of pain

Prevalence of pain, pain characteristics, and use of analgesics

Of the 273 patients included, 246 reported having pain (90%) on the first question of the McGill questionnaire (*Table 1; highlighted row*). Female patients reported pain more frequently than male patients: 92% vs. 74% (P = 0.001), and patients with the hypermobility type EDS reported pain more frequently than those with the classical and vascular type (98% vs. 76% and 55% respectively (P < 0.001); *Table 1*). The findings presented below are the result of the subgroup analyses of these patients with pain (n = 246), while for the multiple regression analysis data of all patients were used (n = 273)(*Figure 1*).

Table 1 Pain severity in the three most common EDS types (n = 246). VAS scores of current, least severe, and most severe pain and use of analgesics of patients with the three most common types of EDS, and differences of pain severity between these types.

EDS type	Total	Classical	Hyper mobility	Vascular	Kypho scoliotic	Not known	Differences between types[1] P-value	
Total number of patients	273	45	162	11	2	53	n.s.	
Prevalence of pain: number of patients	246	34	159	6	1	46	< 0.001	clas < hyper
(% of patients of that type)	(90)	(76)	(98)	(55)	(50)	(87)	< 0.001	hyper > vasc
Joint hypermobility	230	26	157	4	1	42	n.s.	
Dislocations	193	17	137	2	0	37	n.s.	
Dermal features	236	34	149	6	1	46	n.s.	
Muscle weakness	196	27	125	3	0	41	n.s.	
Previous operations	228	33	146	5	1	43	n.s.	
Current pain:	48.0	39.2	49.1	21.2			0.016	clas > vasc
mean VAS score (SD)	(22.4)	(25.4)	(21.0)	(12.0)			0.001	hyper > vasc
Least severe pain:	21.5	19.6	21.6	6.3			0.018	hyper > vasc
mean VAS score (SD)	(16.3)	(17.3)	(15.5)	(6.3)				
Most severe pain:	82.5	76.0	85.3	67.7			0.014	clas < hyper
mean VAS score (SD)	(17.4)	(19.5)	(13.5)	(30.9)				
Use of analgesics:	216	27	145	4			n.s.	
number of patients	(88)	(82)	(91)	(67)				
(% of patients of that type)								

[1] Fisher exact test. n.s.: not significant. -: not tested. clas: classical type EDS; hyper: hypermobility type EDS; vasc: vascular type EDS.

Reported pain severity (VAS of current pain, least severe pain, and most severe pain) is presented in *Table 1*. Overall, patients with the hypermobility type had the highest VAS scores for current pain, least severe pain, and most severe pain (*Table 1*). Pain was most frequently localized in neck, shoulders, hips, and forearms, and legs (> 40% of all patients), which might reflect a pattern of musculoskeletal pain (*Figure 2*). In contrast, headache or abdominal pain was reported infrequently. This was supported by the response to the question of presence of myalgia: frequent or continuous myalgia was reported by 87% of patients with pain. It did not occur in a specific distribution; approximately 60% of patients reported myalgia in arms, legs, and / or trunk.

The majority of patients reported chronic pain (i.e. lasted longer than one year; 92%); gradual increase of pain (84%); and an equal severity of pain (64%). Frequently used adjectives (reported by > 40% of the patients) to characterize the pain were: cutting, nagging, tiring, troublesome, and sickening. Pain severity changed over time, but remained continuously present to some degree in most patients (85%). The majority of patients suffered pain the day before filling out the questionnaire (95%). In 90% of them pain occurred more than 4 hours, and 72% of them had to rest due to pain the previous day. Of the patients resting, 38% rested for at least 4 hours that day. The majority of patients (89%) suffered from pain at the day of filling out the questionnaire, and in 53% of the patients this pain severity was moderate to severe.

One or more analgesics were used by 89% of the EDS patients with pain; paracetamol was used by 59%; paracetamol with codeine by 21%; acetylsalicylic acid by 4%, other non-steroid anti inflammatory drugs by 67%, tramadol by 23%, and anti-neuropathic drugs by 11% of the patients (*Table 2*). Reported doses did not exceed the maximally allowed prescriptions.

The relation of pain with disease-related factors

Joint hypermobility was reported by 230 of the 246 patients with pain. Dislocations occurred in 193 patients, dermal features were reported by 236 patients with pain. Previous surgery was reported by 227 of these patients, and muscle weakness by 196 (*Table 1*).

Most severe pain (VAS) was significantly correlated with previous surgery (0.213; $P < 0.01$), hypermobility (0.175; $P < 0.001$), and dislocations (0.183; $P < 0.001$). Current pain (VAS) was significantly correlated with dislocations (0.153; $P < 0.05$). Neither most severe pain nor current pain (VAS) was significantly correlated with dermal features or muscle weakness.

The relation of pain with sleep disturbances

Half of the patients with pain (n = 246) was awake during the night due to pain (50%); and of these patients, 93% was awake at least two hours. The mean SCL sleep subscale score was 6.7 (SD 3.3; range 3 - 15). Pain severity was significantly correlated with SCL subscale score sleep ($r = 0.31$; $P < 0.001$ for most severe pain, and $r = 0.29$; $P < 0.001$ for current pain $P < 0.001$).

Table 2 Use of analgesics in EDS patients reporting pain (n = 246). Use of analgesics in EDS patients reporting pain; several patients used more than one analgesic.

Drug	% Of patients with pain (n=246) using analgesics
Paracetamol	
Paracetamol	59
Paracetamol with codeine	21
Non-steroidal anti-inflammatory drugs	
Acetylsalicylic acid	4
Ibuprofen	31
Naproxen	14
Diclofenac	16
Opiates	
Tramadol	23
Neuropathic pain drugs	
Amitriptyline	7
Carbamazepine	1
Gabapentin	3
Other	
Various	33

Contribution of pain symptoms to the functional impairment in daily life

Based on the results of the McGill Questionnaire, eighty-seven percent of the patients with pain (n = 246) was impaired in performing their daily activities, and in 55% of them this consisted of moderate to severe impairment. Appetite was reduced in 32% of patients. Furthermore, patients had moderate to severe impairment due to pain based on the SF-36: mean SF-36 sub score pain was 42.4 (range 0 - 100; SD 20.2). Pain severity was significantly correlated with the total SIP score ($r = 0.45$; $p < 0.001$ for most severe pain, and $r = 0.43$ ($P < 0.001$) for current pain). The mean total SIP score was 1157 (SD 660; range 0 - 2865) reflecting severe impairment in daily functioning.

Multiple regression analysis of data of all patients (n = 273) resulted in a model in which pain severity (most severe pain (VAS)) and fatigue severity predicted 31% of functional impairment (*Table 3*). The predictive value of the model with current pain rather than most severe pain as independent variable had a similar predictive value (28%; *data not shown*).

Figure 2 Distribution pattern of pain in EDS patients.

Pain occurred in at least 40% (grey) and respectively 50% (black) of EDS patients. Pain was most frequently localized in neck, shoulders, hips, and forearms, and legs, which might reflect a pattern of musculoskeletal pain.

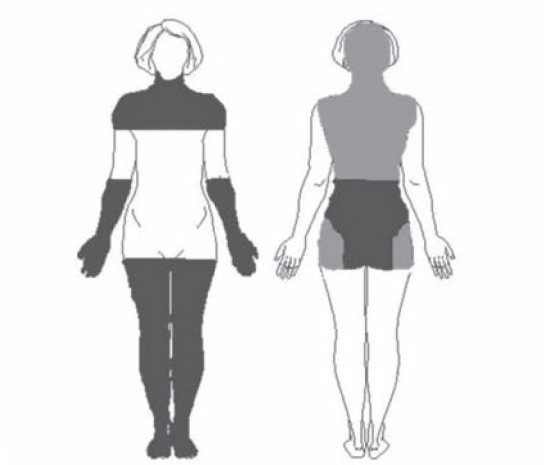


Table 3 Results of multiple regression analysis of pain and fatigue with functional impairment as dependent variables (n = 273). Regression analysis with functional impairment as dependent variable, and pain and fatigue as independent variables.

Dependent variable: Functional impairment (SIP sum score)			
Independent variables		*beta*	*P*
1) Pain	Most severe pain (VAS)	0.241	< 0.001
2) Fatigue	CIS fatigue	0.392	< 0.001

Enter; p in = 0.05; p out = 0.1; adjusted $R^2 = 0.309$.

Discussion

This study in a large group of EDS patients shows that 1) chronic pain is highly prevalent in EDS and is associated with regular use of analgesics; 2) pain is more prevalent and more severe in patients with the hypermobility type than in those with the classical type and vascular type; 3) pain severity is related to hypermobility, dislocations, and previous operations, but not to other disease related factors; 4) pain is related to sleep disturbances; and 5) pain is related to functional impairment in daily life, independently of the level of fatigue.

Previous studies also reported high prevalence of pain in EDS, but were limited in size and scope.[5,7,8] Results of our study confirm the high prevalence of pain and add the finding that pain is most common and most severe in patients with the hypermobility type of EDS. Furthermore, this study shows that myalgia is reported by the majority of patients, and that pain is predominantly localized in neck, shoulders, hips, and legs, but not in head or abdomen. Most severe pain is correlated to hypermobility, dislocations, and previous surgery. Together, these findings may indicate that pain in EDS has a compound origin: a constant level of pain may originate in the musculoskeletal system[1] and additional peaks of severe pain may be related to recurrent (sub)luxations and/or dislocations, as suggested by Sacheti.[5]

The results of this study further show that pain severity in EDS is related to sleep disturbances. Pain has previously been reported as one of the causes of low sleep quality in EDS, causing difficulties in initiating and maintaining sleep.[13] Also in other chronic diseases, pain severity was found to be related to sleep disturbances. However, recent data seem more consistent with poor sleep leading to an increasing pain severity than pain predicting poor sleep.[25] Furthermore, pain severity in EDS was found to be independently related to functional impairment. This relationship between pain severity and disability has also been found in other populations with chronic pain, e.g. in patients with chronic lower back pain or fibromyalgia.[25]

This study has not focused on the psychological variables which are known to influence pain, such as catastrophizing of pain and fear of exercise-related pain.[14] These and other factors should be addressed in future studies on pain in EDS, since they may be a starting point for treatment of chronic pain in EDS.[26,27] Based on experience with chronic pain in other diseases, cognitive behavioural interventions in EDS might reduce pain and pain related disability.[26] In addition, symptomatic treatment of pain in EDS can be directed at prevention of dislocations and optimizing medical treatment of pain. Patients' report of severe pain in EDS stress the importance of development of multidisciplinary treatment protocols.[6]

A possible limitation of our study is the selection bias occurring with recruitment of members of the Dutch EDS Foundation. The previous questionnaire study on 51 patients, which showed that moderate to severe pain is common in EDS, also relied on members of a

national EDS foundation.[5] Patients who are most severely affected might be more likely to join a patient support group. EDS patients with only mild symptoms might not even be diagnosed and not seek medical attention. This selection bias has to be taken into account when interpreting the results of this study.

These limitations notwithstanding, our findings suggest that pain is a very common and severe symptom in this group of EDS patients. It is related to dislocations, sleep disturbances, and moderate to severe impairment in daily functioning. Therefore, treatment of pain should be a prominent aspect of clinical management of EDS and multidisciplinary protocols should be developed.

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
3. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
4. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.
5. Sacheti A, Szemere J, Bernstein B, Tafas T, Schechter N, Tsipouras P. Chronic pain is a manifestation of the Ehlers-Danlos syndrome. J Pain Symptom Manage 1997; 14: 88-93.
6. Gawthrop F, Mould R, Sperritt A, Neale F. Ehlers-Danlos syndrome. BMJ 2007; 335: 448-450.
7. Berglund B, Nordstrom G. Symptoms and functional health status of individuals with Ehlers-Danlos syndrome (EDS). J Clin Rheumatol 2001; 7: 308-314.
8. Lumley MA, Jordan M, Rubenstein R, Tsipouras P, Evans MI. Psychosocial functioning in the Ehlers-Danlos syndrome. Am J Med Genet 1994; 53: 149-152.
9. Berglund B, Nordstrom G, Hagberg C, Mattiasson AC. Foot pain and disability in individuals with Ehlers-Danlos syndrome (EDS): impact on daily life activities. Disabil Rehabil 2005; 27: 164-169.
10. Baakman WBE. [Quality of life in patients with Ehlers-Danlos syndrome]. Utrecht: University Utrecht, Health Psychology; 2002.
11. Voermans NC, Knoop H, van de KN, Hamel BC, Bleijenberg G, van Engelen BG. Fatigue Is a Frequent and Clinically Relevant Problem in Ehlers-Danlos Syndrome. Semin Arthritis Rheum 2009.
12. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
13. Verbraecken J, Declerck A, van de Heyning P, de Backer W, Wouters EF. Evaluation for sleep apnea in patients with Ehlers-Danlos syndrome and Marfan: a questionnaire study. Clin Genet 2001; 60: 360-365.
14. Keefe FJ, Rumble ME, Scipio CD, Giordano LA, Perri LM. Psychological aspects of persistent pain: current state of the science. J Pain 2004; 5: 195-211.
15. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1975; 1: 277-299.
16. Scheeres K, Wensing M, Severens H, Adang E, Bleijenberg G. Determinants of health care use in chronic fatigue syndrome patients: a cross-sectional study. J Psychosom Res 2008; 65: 39-46.
17. Arrindell WA, Ettema JHM. SCL-90-R: Handleiding bij een multidimensionele psychopathologie-indicator. Lisse: Swets & Zeitlinger; 1986.
18. Ware JE, Snow KK, Kosinski M. SF-36 Health Survey Manual and Interpretation Guide. Boston, MA: New England Medical Center, The Health Institute. 2008.
19. Stewart JW, Quitkin FM, McGrath PJ, Rabkin JG, Markowitz JS, Tricamo E, Klein DF. Social functioning in chronic depression: effect of 6 weeks of antidepressant treatment. Psychiatry Res 1988; 25: 213-222.
20. Berger F, Brahler E, Kunkel R, Stephanos S. [Verbal behavior and communication experience of psychosomatic patients in the first psychoanalytic interview in connection with the concept of "pensee operatoire"]. Z Psychosom Med Psychoanal 1981; 27: 45-58.
21. Jacobs HM, Luttik A, Touw-Otten FW, de Melker RA. [The sickness impact profile; results of an evaluation study of the Dutch version]. Ned Tijdschr Geneeskd 1990; 134: 1950-1954.
22. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. The development of a model of fatigue in neuromuscular disorders: a longitudinal study. J Psychosom Res 2007; 62: 571-579.
23. Pfeiffer G, Wicklein EM, Ratusinski T, Schmitt L, Kunze K. Disability and quality of life in Charcot-Marie-Tooth disease type 1. J Neurol Neurosurg Psychiatry 2001; 70: 548-550.
24. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. J Psychosom Res 1994; 38: 383-392.

25. Naughton F, Ashworth P, Skevington SM. Does sleep quality predict pain-related disability in chronic pain patients? The mediating roles of depression and pain severity. Pain 2007; 127: 243-252.
26. Morley S, Eccleston C, Williams A. Systematic review and meta-analysis of randomized controlled trials of cognitive behaviour therapy and behaviour therapy for chronic pain in adults, excluding headache. Pain 1999; 80: 1-13.
27. Morley S, Williams A, Hussain S. Estimating the clinical effectiveness of cognitive behavioural therapy in the clinic: evaluation of a CBT informed pain management programme. Pain 2008; 137: 670-680.

PART II

Neuromuscular features of Ehlers-Danlos syndrome

B

Quantitative muscle function measurements of tenascin-X-deficient Ehlers-Danlos syndrome patients and *tenascin-XB* knockout mice

CHAPTER 7

Reduced quantitative muscle function in tenascin-X-deficient Ehlers-Danlos patients

Adapted from:
Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG.
Neuromuscul Disord. 2007;8:597-602.

Abstract

The Ehlers-Danlos Syndrome (EDS) is a heterogeneous group of inherited connective tissue disorders. Skeletal muscle features belong to the clinical criteria of EDS and are generally interpreted to result from increased tendon distensibility or exercise avoidance. However, muscle function in EDS has hardly been investigated as such.

We performed a pilot study consisting of clinical investigations, electromyography, muscle ultrasound, muscle biopsy, and quantitative muscle function tests on two EDS patients with deficiency of tenascin-X. Quantitative muscle function proved severely reduced despite normal findings on electromyography and muscle biopsy. These findings dispute the interpretation of increased tendon distensibility. We hypothesize that alterations in the extracellular matrix modify myofascial force transmission and thus influence muscle function in EDS.

Introduction

The Ehlers-Danlos Syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders (ICTDs) characterized by articular hypermobility, skin hyperextensibility and tissue fragility.[1] Mutations in type V collagen can explain part of classical type EDS cases.[1] A clinically distinct, recessive form results from tenascin-X (TNX) deficiency.[2] The hypermobility type of EDS has been associated with TNXB gene haploinsufficiency.[3]

The diagnostic criteria of EDS include muscle hypotonia, and muscle symptoms have been reported.[1,4-7] Muscle symptoms are generally interpreted to result from increased distensibility of tendons or from exercise avoidance due to joint hypermobility and pain.[7] However, muscle function in EDS has hardly been investigated as such. We therefore performed a pilot study with two EDS patients comprising clinical investigations, electromyography, muscle ultrasound, muscle biopsy, and quantitative muscle function tests.

Methods

Patient selection

We selected two female EDS patients: one with TNX deficiency and absence of TNX in serum, with a clinical presentation resembling the classical type of EDS. She has been previously been described by Schalkwijk et al. in 2001 (*Patient 1*)[2] and by us in 2007[8] (3rd Report in *Chapter 3*); and one with hypermobility type of EDS with reduced TNX levels in serum probably due to *TNXB* gene haploinsufficiency.[3] Mutation analysis of *TNXB* in patient 1 revealed a homozygous 2-bp deletion in exon 8 that encodes the fourth fibronectin type III repeat. Analysis of the mutations described by Schalkwijk et al. in patient 2 revealed no abnormalities.[2]

Clinical studies

Muscle ultrasound

Muscle ultrasound of the quadriceps muscle was performed using standard techniques as previously described.[9] Results were compared with normal values acquired in healthy adults in the same centre.[10]

Electromyography

Electrophysiological examination was performed using standard techniques with a Medelec Synergy EMG system (Oxford Medical Instruments, Surrey, United Kingdom).

Muscle biopsy

Muscle biopsy specimens were obtained from the right vastus lateralis muscle in both

patients. Frozen sections of 10 µm were examined using standard enzyme histochemical and immune histochemical techniques. Guinea pig anti-human TNX antibodies were used to stain TNX within muscle ECM.[2]

Quantitative muscle function testing and analysis

In a single experimental session the patients' neuromuscular function of the quadriceps muscle was investigated as reported previously.[11] They were asked to perform and sustain a maximal voluntary contraction during approximately three seconds. Next, a 10 Hz contraction was elicited with seven stimulation pulses and a fused tetanic contraction was evoked with 150 Hz stimulation for one second. Isometric knee extension torque was measured at 90-degree knee flexion with the subjects seated on a custom-built rigid chair. Torque was measured with a force transducer fixed to the shin. During each voluntary contraction the subjects were vigorously verbally encouraged, and visual feedback allowed them to achieve maximal performance. Torque signals were digitized (1000 Hz) and stored on disc for immediate and off-line analysis with custom Matlab software packages. From the signals, maximal rates of torque generation and relaxation were calculated, as well as the time between the stimulus and initial torque rise. Results were compared with previously published normal values.[12,13]

Results

Patient 1

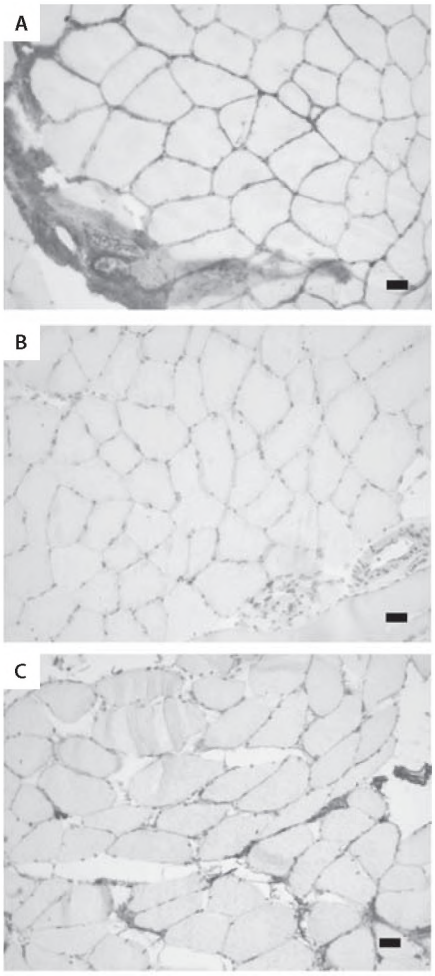
A 46-year-old woman with TNX-deficient type of EDS presented with weakness of her right hand and mild generalized weakness of arms and legs, which had started insidiously four years prior to testing. She had suffered from recurrent dislocations of her right shoulder, was unable to walk up stairs and had difficulties swallowing. Her walking endurance was limited to one hour and she had difficulties keeping her balance on uneven ground. A general physical examination revealed hypermobility, bruises on her fingers, bilateral pes planus, and hyperextensibility of her skin, which was smooth and velvety. The results of the neurological examination included mild dysphagia, generalized muscle hypotonia, mild weakness of the neck flexor muscles and the arms and legs, both proximally and distally, with the most pronounced weakness in the muscles of her right hand. There was muscle atrophy of her right lower arm and hand. Tendon reflexes were symmetrically low *(Table 1)*.

Nerve conduction studies were normal and electromyography showed mild aspecific changes, without clear signs of either a myopathy or neurogenic disorder. Muscle ultrasound of the quadriceps muscle revealed no atrophy. Muscle biopsy of the quadriceps muscle showed no significant myopathic changes. Staining with antibodies against TNX revealed absence of TNX within muscle ECM (*Figure 1*). Quantitative muscle function testing (*Table 2*)

revealed a low maximal knee extension torque at voluntary contraction. During voluntary effort, maximal performance was only achieved after practice and with verbal encouragement. Torques were most steady with visual feedback (*Figure 2*). The torque variation with 10 Hz stimulation looked normal. However, the twitch torque was relatively high. The interval between stimulation and initial torque production, as well as the relaxation pattern were normal. Torque generation at 150 Hz appeared faster than normal (*Figure 3*).

Figure 1 Muscle biopsies stained with anti-human TNX antibodies: qualitative evaluation of staining. Bar = 50 µm.

A: The biopsy of a control subject: normal TNX staining. **B:** The biopsy of patient 1: absence of TNX. **C:** The biopsy of patient 2: reduced staining of TNX.



Patient 2

A 22-year-old woman with EDS of the hypermobility type with reduced TNX serum levels had suffered from muscle hypotonia since infancy. Her gross motor development had been delayed and she had never been proficient at sports. Her family history was positive for EDS of the hypermobility type. She had received two corrective surgeries for her congenital hip dysplasia but still experienced difficulty walking, which is why she had been using a wheelchair for the preceding three years. She played wheelchair tennis twice a week for 30 minutes. She reported experiencing gradual, progressive muscle weakness, which was more pronounced in her legs than in her arms, and occasional muscle cramps in her calves.

The general physical examination revealed hypermobility and mild scoliosis. There was no hyperextensibility of the skin but scars were broad, striae were present on abdomen and hips, and the skin was smooth and velvety. Neurological examination revealed a mild paresis of the right proximal arm muscles and of both legs with generalized hypotonia. Muscle atrophy was pronounced in her right arm and right anterior tibial muscle, but absent in her quadriceps muscles. She was confined to a wheelchair and was only able to remain upright without support for a short period. Tendon reflexes were symmetrically low (*Table 1*).

Table 1 Results of physical examination.

	Patient 1		Patient 2	
Beighton score	5/9		5/9	
Skin features	bruises on fingers; hyperextensibility; smooth, velvety skin		broad scars; striae on abdomen and hips; no hyperextensibility; smooth, velvety skin	
Muscle tone	generalized hypotonia		generalized hypotonia	
Muscle atrophy	none		atrophy of right arm muscles and of right anterior tibial muscle	
Deep tendon reflexes	symmetrically low		symmetrically low	
Sensation	normal		normal	
Muscle strength (MRC)	Right	Left	Right	Left
Neck flexors	4		4	
Biceps brachii	4	4	4	5
Flexion wrist	4	4	5	5
Deep flexors of fingers	3	4	5	5
Iliopsoas	4	4	3	4
Quadriceps	5	5	4	4
Anterior tibial	4	4	3	4

MRC: Medical Research Council.[14]

Results of nerve conduction studies, electromyography and muscle ultrasound were normal. A needle biopsy of the quadriceps muscle showed no significant myopathic changes. Staining with antibodies against TNX revealed reduced presence of TNX within muscle ECM (*Figure 1*). The torque characteristics of this patient were very similar to those of patient 1 (see *Table 2* and *Figure 2*): a low maximal torque, a relatively high twitch torque, a normal interval between stimulation and initial torque production, a normal relaxation, and relatively fast torque generation. Again, maximal performance during voluntary effort was only achieved after practice and visual feedback enhanced steadiness of the torque.

Table 2 Results of ancillary investigations.

	Patient 1	Patient 2	Normal values
Clinical test			
Electromyography	Mild aspecific changes: some small, polyphasic units	Normal	
Muscle ultrasound of the quadriceps muscle	Normal	Normal	
Needle biopsy of the quadriceps muscle	No significant myopathic changes	No significant myopathic changes	
Type I fibres: Percentage and diameter	60% 46 - 92 µm	24% 46 - 63 µm	35 - 50% 40 - 84 µm
Type II fibres: Percentage and diameter	40% 50 - 83 µm	76% 46 – 63 µm	50 - 65% 40 - 84 µm
Quantitative muscle function testing			
Maximal knee extension torque at voluntary contraction (Nm)	87	74	125 ± 23 (n = 8)[13]
Torque variation with 10 Hz stimulation	normal	normal	
Twitch torque	26% of tetanic torque	27% of tetanic torque	19 ± 2% of tetanic torque (n = 8)[13]
Interval between stimulation and initial torque production	normal	normal	
Relaxation pattern	normal	normal	
Rate of torque generation at 150 Hz (%Tmax/ms)	1.60%	1.72%	1.43 ± 0.02% (n = 8)[13]

Figure 2 Torque production over time during repeated maximal voluntary attempts with visual feedback and verbal encouragement, showing torque increases after serial attempts with visual feedback.

A: For patient 1 the first, second and fifth attempt are depicted in the black, dark grey and light grey curves, respectively. **B:** For patient 2 the torque productions of the first, third and sixth attempt are shown in the thick, medium and thin light grey curves, respectively.

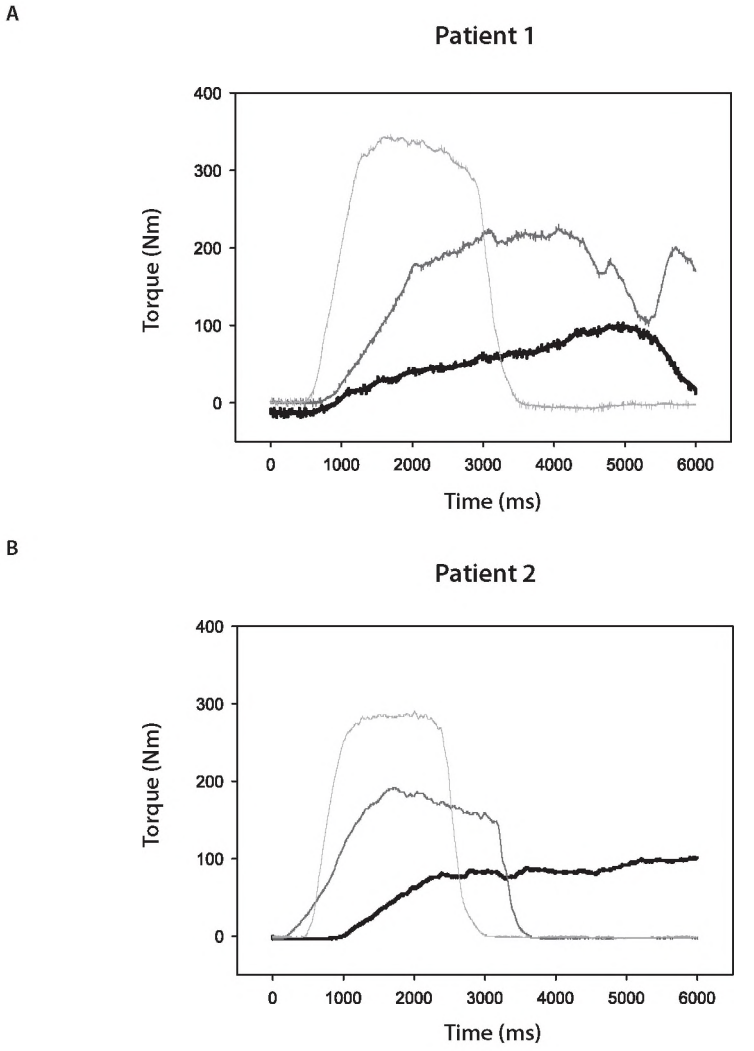
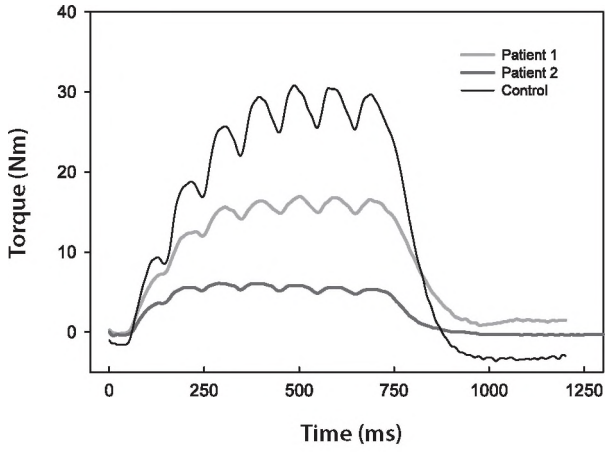


Figure 3 Torque production as a result of 10 Hz stimulation for patient 1, patient 2 and a healthy subject, showing reduced torque production, but normal torque variation.



Discussion

The main finding of our study is that quantitative muscle function proved severely reduced in the two EDS patients, despite normal findings on electromyography and muscle biopsy. Our data are compatible with the one case study that addressed muscle function in the classical type of EDS in which generalized mild to moderate weakness was observed.[15]

It is unlikely that enhanced tendon compliance due to increased tendon distensibility can account for the observed muscle weakness in this study. First, increased tendon compliance would have increased the interval between stimulation and initial torque production and would have affected the relaxation rate (this is mostly pronounced at short muscle length), which were both normal in both patients. Second, it would have slowed torque generation whereas in these patients it was accelerated. Furthermore, increased compliance is assumed to decrease efficiency of contraction, forcing muscle cells to operate at a relatively shortened length, which would result in lower twitch torques. Yet, both patients displayed relatively high twitch torques. Thus, although TNX is expressed in tendon sheaths, changes in myotendinous force transmission cannot explain the present results.

Furthermore, disuse cannot fully account for the results of this study, since torque generation would then be relatively faster, and relative twitch torques would be higher than in control subjects. Our results did not show this. First, the findings in both patients were very

similar, whereas patient 1 was normally ambulant. Second, physical examination, muscle ultrasound, and biopsy revealed no signs of atrophy in the quadriceps muscle in either of the patients. Fibre size diameter was within the normal range in both patients, but slightly higher in patient 1 than in patient 2. This difference might correspond to the somewhat higher maximal knee extension torque at voluntary contraction in patient 1, and reflects the limited contribution of disuse to muscle weakness in patient 2.

Alternatively, since TNX is expressed in muscle ECM in animal models,[16-19] absence or reduction of TNX in the ECM of muscle may influence muscle function in these EDS patients. Hence, rather than in its adjacent structures (tendons and joints), the cause of muscle weakness in EDS may be located in the ECM of muscle itself. The ECM of skeletal muscles is located between muscle fibres (endomysium), surrounds fascicles (perimysium), and covers the whole muscle (epimysium). It shapes the muscle and gives the contractile cells cohesiveness and elasticity, and has additional functions at the neuromuscular and myotendinous junctions. TNX indeed plays a central role in the organization of muscle ECM through interaction with various ECM components, such as collagen I, III, V, XII and XIV. Consequently, TNX might be important for the compliance of connective tissues.[16]

Previous studies on the role of the muscle ECM in muscle function have lead to the concept of myofascial force transmission. It is generally assumed that muscle force is transmitted via the myotendinous junction to the tendon and further onto the bone. However, in addition to *myotendinous* force transmission, up to 40% of muscle force can be transmitted from muscle fibres onto the endomysium and from there to peri- and epimysium, and extra muscular connective tissue.[20] This so-called *myofascial* force transmission might be influenced by altered tensile characteristics of muscle ECM as in EDS. Hence, absence or reduced presence of TNX in muscle ECM in these patients, as shown in *Figure 1*, may point to a relation between reduced myofascial force transmission and muscle weakness in EDS. The exact mode in which the TNX-deficient ECM affects muscle function has neither been investigated in this study nor in previous studies. However, it is conceivable that TNX deficiency reduces the stiffness of epimuscular myofascial pathways and thus causes a pathological reduction of the force transmitted this way. This would change the required muscular coordination drastically and interfere with mechanical interaction between antagonistic muscles.[21]

In conclusion, quantitative muscle function proved severely reduced in the two EDS patients, despite normal findings on electromyography and muscle biopsy. These finding dispute the common interpretation of increased tendon distensibility, and support the hypothesis that alterations in the ECM influence muscle function in EDS.

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
3. Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M, Kucharekova M, Boezeman JB, Schalkwijk J. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet 2003; 73: 214-217.
4. Beighton P, Price A, Lord J, Dickson E. Variants of the Ehlers-Danlos syndrome. Clinical, biochemical, haematological, and chromosomal features of 100 patients. Ann Rheum Dis 1969; 28: 228-245.
5. Pretorius ME, Butler IJ. Neurologic manifestations of Ehlers-Danlos syndrome. Neurology 1983; 33: 1087-1089.
6. Banerjee G, Agarwal RK, Shembesh NM, el Mauhoub M. Ehlers Danlos syndrome--masquerading as primary muscle disease. Postgrad Med J 1988; 64: 126-127.
7. Steinmann B, Royce PM, Superti-Furga A. The Ehlers-Danlos syndromes. In: Steinmann B, Royce P.M., editors. Connective Tissue and Its Heritable Disorders. Wiley-Liss Inc.; 2002. p. 431-523.
8. Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Ehlers-Danlos syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies. Am J Med Genet A 2007; 143: 2215-2219.
9. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. Muscle Nerve 2003; 27: 693-698.
10. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
11. Gerrits K, Gommans I, van Engelen B, de Haan A. Quadriceps weakness in a family with nemaline myopathy: influence of knee angle. Clin Sci (Lond) 2003; 105: 585-589.
12. Bazzucchi I, Felici F, Macaluso A, De Vito G. Differences between young and older women in maximal force, force fluctuations, and surface EMG during isometric knee extension and elbow flexion. Muscle Nerve 2004; 30: 626-635.
13. de Haan A, de Ruiter CJ, van der Woude LH, Jongen PJ. Contractile properties and fatigue of quadriceps muscles in multiple sclerosis. Muscle Nerve 2000; 23: 1534-1541.
14. Peterson-Kendall F, Kendall-McCreary E, Geise-Provance P, McIntyre-Rodgers M, Romani WA. Muscles testing and Function with Posture and Pain. Baltimore, MD, USA: Lipincott Williams & Wilkins; 2005.
15. Bilkey WJ, Baxter TL, Kottke FJ, Mundale MO. Muscle formation in Ehlers-Danlos syndrome. Arch Phys Med Rehabil 1981; 62: 444-448.
16. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. J Pathol 2003; 200: 423-428.
17. Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. Dev Dyn 1995; 203: 491-504.
18. Veit G, Hansen U, Keene DR, Bruckner P, Chiquet-Ehrismann R, Chiquet M, Koch M. Collagen XII interacts with avian tenascin-X through its NC3 domain. J Biol Chem 2006; 281: 27461-27470.
19. Lethias C, Carisey A, Comte J, Cluzel C, Exposito JY. A model of tenascin-X integration within the collagenous network. FEBS Lett 2006; 580: 6281-6285.
20. Huijing PA. Muscular force transmission necessitates a multilevel integrative approach to the analysis of function of skeletal muscle. Exerc Sport Sci Rev 2003; 31: 167-175.
21. Huijing PA. Epimuscular myofascial force transmission between antagonistic and synergistic muscles can explain movement limitation in spastic paresis. J Electromyogr Kinesiol 2007; 17: 708-724.

CHAPTER 8

Mild muscular features in tenascin-XB knockout mice, a model of Ehlers-Danlos syndrome

Adapted from:
Voermans NC, Verrijp K, Eshuis L, Balemans MCM, Egging DF, Sterrenburg E, van Rooij IALM, van der Laak JAWM, Schalkwijk J, van der Maarel SM, Lammens M, van Engelen BG.
Connect Tissue Res 2011 Mar 15 [Epub ahead of print]

Abstract

Tenascin-X (TNX) is an extracellular matrix glycoprotein whose absence in humans leads to a recessive form of Ehlers-Danlos Syndrome (EDS), a group of inherited connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. A mouse model of the TNX-deficient type EDS has been used to characterize the dermatological, orthopaedic, and obstetrical features. The growing insight in the clinical overlap between myopathies and inherited connective tissue disorders asks for a study of the muscular characteristics of inherited connective tissue diseases. Therefore, this study aims to define the muscular phenotype of *Tnxb* knockout mice.

We performed a comprehensive study on the muscular phenotype of these *Tnxb* knockout mice, consisting of standardized clinical assessment, muscle histology, and gene expression profiling of muscle tissue. Furthermore, peripheral nerve composition was studied by histology and electronmicroscopy.

The main findings are presence of mild muscle weakness, mild myopathic features on histology, and functional upregulation of genes encoding proteins involved in extracellular matrix degradation and synthesis. Additionally, sciatic nerve samples showed mildly reduced collagen fibril density of endoneurium.

The muscular phenotype of *Tnxb* knockout mice consists of mild muscle weakness with histological signs of myopathy and of increased turnover of the extracellular matrix in muscle. Furthermore, mildly reduced diameter of myelinated fibres and reduction of collagen fibril density of endoneurium may correspond with polyneuropathy in TNX-deficient EDS patients. This comprehensive assessment can serve as a starting point for further investigations on neuromuscular function in *Tnxb* knockout mice.

Introduction

Tenascin-X (TNX) is an extracellular matrix (ECM) glycoprotein, the absence of which in humans leads to a recessive form of Ehlers-Danlos Syndrome (EDS), a group of related inherited connective tissue disorders.[1,2] TNX-deficient type EDS has a phenotype similar to the classic type EDS with hypermobile joints, hyperextensible skin, and easy bruising, but without atrophic scarring and with an autosomal recessive inheritance pattern.[1,3] Haploinsufficiency of *Tenascin-XB* (*TNXB*) is associated with joint hypermobility in females.[4]

Tnxb knockout (KO) mice have been used for detailed study of the dermatological phenotype and to increase insight in the dermatological features of TNX-deficient type EDS.[5-8] Additionally, articular and obstetrical aspects were investigated, the results of which showed that this mouse model only partly reflects the multisystem involvement of TNX-deficient type of EDS in human.[9,10] Protein expression studies in *Tnxb* KO mice tissue have further suggested a role of TNX in developing and adult muscles; TNX was found to be abundantly expressed in various tissues during embryonic development, among which are perimysium of skeletal muscle and tendons.[11,12] In adulthood, TNX is predominantly expressed in skeletal and cardiac muscle. Therefore, TNX was initially referred to as 'the muscle tenascin'.[12] In addition, in both patients and mice, abnormalities of elastic fibres were observed in skin.[13] TNX was indeed found to be highly expressed in the peripheral nervous system, more specifically in perineurium and endoneurium.[14,15] However, myelin sheath thickness, axonal size, and ultrastructure of the sciatic nerve of *Tnxb* KO mice were reported to be normal.[15] Hence, it was suggested that TNX has only a subtle function in peripheral nerve macromolecular organization.[15]

The growing insight in the clinical overlap of myopathies and inherited connective tissue disorders such as collagen VI myopathies, EDS, and Marfan syndrome[16-18] calls for a study of the muscular phenotype of inherited connective tissue disorders. In the initial dermatological study, *Tnxb* KO mice were reported to have no perinatal abnormalities, exhibit normal growth, and develop progressive skin hyperextensibility, similarly as in EDS patients.[5] Therefore, *Tnxb* KO mice probably offer a suitable animal model to study the muscular phenotype of TNX-deficient EDS in more detail. Despite the abovementioned histological findings, the muscular phenotype of *Tnxb* KO mice has not been investigated in detail so far.

This study aims to assess the muscular phenotype of *Tnxb* KO mice in a broad approach, consisting of standardized clinical assessment, muscle histology, gene expression profiling on muscle tissue, and peripheral nerve histology and electronmicroscopy. Such detailed observation will enable a comprehensive assessment of the muscular phenotype of *Tnxb* KO mice, which may be a good starting point for further investigations on the influence of alterations of the ECM on muscle and peripheral nerve function. Additionally, it may increase insight in the neuromuscular symptoms in TNX-deficient EDS patients and strengthen the connection between the ECM and neuromuscular features.[19]

Materials and methods

Mice

Tnxb KO mice were obtained from Bristow et al.[3] These mice have a heterogeneous genetic background. Therefore, mice backcrossed to a C57/BL6 background, as described previously[5,9] were used in these studies. Briefly; -/- offspring from heterozygous parents was obtained by the backcrossing of *Tnxb* KO mice with six generations of C57/BL6 mice. Wild-type (WT) C57/BL6 mice were used as controls. Long term motor activity assessment was performed in eight female *Tnxb* KO mice and eight female WT mice, both at six and 16 months. For functional strength measurements, eight female *Tnxb* KO mice and eight female WT mice were tested at the age of three, six, and 16 months. Body weight was measured at 16 months of age. Muscle biopsies of the quadriceps muscles for histochemical studies were obtained in six male *Tnxb* KO and WT mice of eight months of age. Gene expression studies were performed on biopsies of the quadriceps muscle of the same male *Tnxb* KO and WT mice of eight months of age. Since we detected the presence of axonal sensomotor polyneuropathy in TNX-deficient EDS patients,[20] we made a histological and electronmicroscopic analysis of the sciatic nerve of one *Tnxb* KO and one WT mouse of 16 months of age, both females. The experimental design was approved by the animal use committee of Radboud University Nijmegen Medical Centre.

Long term motor activity assessment

Straightforward observation of motor behaviour of *Tnxb* KO mice and WT mice revealed no differences.[5] We therefore assessed long term motor activity in a home cage-like environment ('Phenotyper') as previously reported.[21,22] *Tnxb* KO and WT mice (n = 8 in each group; female; 6 and 16 months) were randomly placed in 16 cages with versatile video-based observation system for one week (Noldus PhenoTyper, Wageningen, The Netherlands). We used the mobility detection parameter of the Ethovision video tracking software (Noldus; immobility threshold setting of 20% and strong mobility threshold of 60%) to measure the level of activity. This was expressed as mean hourly distance moved (cm) at six and 16 months of age. Additionally, we measured the mean duration of immobility (s), total duration of mobility (s), and total duration of strong mobility (s) per hour at 16 months of age. Data were recorded digitally and quantified automatically as previously described.[21,22]

Functional muscle strength measurement

Functional muscle strength was tested with use of a paw-fall-through test and hang-time test.[23] Mice (n = 8 in each group; female; 3, 6, and 16 months) were placed on a 60 x 40 cm piece of 1.5-cm mesh hardware cloth, approximately 35 cm above their cage filled with sawdust. During a 1-minute observation period, the number of times individual limbs fell

through the wire was counted (number of paw-fall-through events in 1 min: 'PFT 1min'). The network was then inverted and the time for the mouse to fall off was recorded during a maximum of two minutes before being placed back into its cage (hangtime duration: 'HT dur' (ms)).

Quadriceps muscle samples

At 8 months of age 6 male *Tnxb* KO mice and 6 male WT littermates were sacrificed by cervical dislocation. The quadriceps femoris muscle samples were obtained bilaterally immediately after death. The material was snap-frozen in chilled isopentane at - 140 °C. Sections of 10 µm underwent Hematoxylin-Phloxine staining and were examined under a light microscope. Of each mouse, both left and right sections were studied. Presence of fibrosis was evaluated qualitatively. The number of cells with internal nuclei was measured by evaluating a minimal of 300 fibres of each biopsy, and calculating the percentage of cells with internal nuclei. Up to 3 to 5% of cells with internal nuclei can be considered normal. A higher percentage of internal nuclei is indicative for myopathy. Fibre diameters were measured in both WT and *Tnxb* KO mice using KS400 image analysis software (Zeiss GMBH, Germany). An interactive digital region growing procedure with possibility for interactive correction was applied to recognize individual muscle fibres. Subsequently, the software calculated the diameter of each muscle fibre automatically. Diameters were converted from pixels to micrometers.[24]

Subsequently, immunohistochemical staining with antibodies to collagen I, III, V, VI, TNX, elastin, and laminin α2 was performed. Collagen I, III, and V were selected since they are known to be deficient in the classic or vascular type EDS, collagen VI since it is deficient in two myopathies (Ullrich congenital muscular dystrophy and Bethlem myopathy) with clinical features overlapping those of EDS,[25] and elastin since it is involved in cutis laxa, another inherited connective tissue disorder with muscle weakness. Laminin α2 antibodies were included as a control staining. The following antibodies were used: polyclonal goat anti-collagen I antibodies (2 µg/ml), polyclonal goat anti-collagen III antibodies (2 µg/ml), polyclonal goat anti-collagen V antibodies (Southern Biotech, USA) (2 µg/ml), monoclonal mouse anti human collagen VI antibodies (Chemicon international, California, USA), polyclonal goat anti-rat elastin antibodies (Elastin Products Company, USA), and polyclonal rabbit anti-laminin α2 antibodies (DAKO Cytomation, Denmark) (2 µg/ml), and polyclonal rabbit anti-TNX antibodies.[9] Frozen sections were fixed in ice-cold acetone for 10 minutes, dried and incubated with the primary antibody diluted in PBS containing 1 % BSA overnight at 4 degrees. After washing with PBS, the bound antibodies were detected with a biotinylated secondary antibody (Vector, Burlingame, CA). After using the Vectastain Elite ABC kit the sections were coloured with AEC Chromogen (ScyTek labatories, USA). Sections were counterstained with Hematoxylin. Staining of these proteins in endomysium and perimysium was individually scored by two investigators and graded in five semi-quantitative categories:

0 = absence; 1 = limited staining; 2 = moderate staining; 3 = considerable staining; 4 = strong staining. Subsequently, consensus was reached in case of unequal scores. The scores were averaged to reach an overall score for KO and WT mice based upon these individual semi-quantitative scores.

Gene expression profiling

Total RNA was isolated from quadriceps muscle of WT and *Tnxb* KO mice (8 months of age) as described.[26] cRNA was prepared by linear amplification and concurrent incorporation of biotin-UTP with the TotalPrep RNA amplification kit from Ambion, USA. Quantity and quality of the cRNA was checked with the Bioanalyzer lab-on-a-chip. 1.5 µg Of cRNA from each sample was hybridized to the Illumina Mouse Sentrix-6 BeadChip. Arrays were normalized and analyzed with Rosetta Resolver.

Sciatic nerve sample

The sciatic nerve was obtained bilaterally immediately after death (n = 1 in each group; female; 16 months). The proximal part of the nerve was snap-frozen in chilled isopentane at - 140 °C, and the distal end was fixated in 2% Glutaraldehyd in 0.1M Na-cacodylate, postfixed in 1% K-hexacyanoferrat(II).3H20 in 1% Osmium Tetroxide - stained with 4% uranylacetate for 30 min. and lead citrate for 10 min. For each mouse, images of ten representative electron microscopic fields of view were recorded using a JEM-1200EX II (Jeol Europe B.V., The Netherlands) microscope at low magnification setting (1.2k – 1.5k). Image acquisition was performed using digital imaging plate technology for TEM via the Dibis Micron Vario (Ditabis, Pforzheim, Germany). This system uses reusable image plates which are exposed in the TEM identical to classical photo negatives. Plates were read out digitally in a separate system (read out pixel size 17.5 x 17.5 µm^2). Resulting images (size 4910 x 4340 pixels) were stored as uncompressed tiff files. An image of a carbon replica specimen (2160 lines/mm line replica; EMS, Hatfield, UK) was recorded prior to acquisition of images for each mouse. Images were analyzed using KS400 image analysis software (Zeiss GMBH, Germany). This software uses an adaptive digital region growing algorithm to automatically recognize individual myelinated axons. The inner and outer diameter of each myelinated axon was measured as described previously for the muscle fibres; this implies measurement of the minor axis of the ellipse as the diameter.[24]

Statistics

The behavioral data (long term motor assessment and function muscle strength measurement) were recorded in a SPSS database (SPSS version 16.0, SPSS Inc, Chicago, IL, USA). Statistical analyses were performed using a Student's t-test for normally distributed continuous variables and a Mann-Whitney U test for skewed continuous variables.

Differences in density of staining of the muscle biopsies with the various antibodies were calculated with the Mann-Whitney-U test. For the analysis of muscle fibre diameters, histograms were produced. The 5th and 95th percentiles of muscle fibre diameter in WT mice were calculated. Subsequently, the percentage of fibres below this 5th and exceeding the 95th percentiles were determined for each mouse biopsy. Percentages in *Tnxb* KO mice were compared with WT mice (Mann-Whitney U-test).

Histograms of myelinated fibre diameters were produced. Significance of observed differences between distributions were calculated using the non-parametric two-sample Kolmogorov-Smirnov test and the Mann-Whitney U test (central tendency). To find differentially expressed genes ANOVA analyses were performed on the gene expression data in Rosetta Resolver v4 ($P < 0.001$, Bonferroni correction). Differentially expressed genes were exported and, functional annotation was determined using WebGestalt software.[27] A hypergeometric test was performed with the whole gene list as a reference ($P < 0.01$) to find functional groups that were overrepresented in this gene list. Biosemantic analysis was performed using Anni 2.0 to find other disorders in which similar genes were shown to be involved in the disease mechanism as in the *Tnxb* KO mice.[28]

Results

Long term motor activity assessment

As expected, long term assessment of motor activity showed the longest distance moved during the first night and day, and an obvious day-night rhythm for all parameters. Although the *Tnxb* KO mice tended to move more at nighttime, no statistically significant differences between de WT and *Tnxb* KO mice in hourly walking distance, duration of immobility, duration of mobility, or duration of strong mobility were found at six or 16 months of age (*Figure 1*).

Functional muscle strength measurement

Tnxb KO mice showed statistically significantly more paw-fall-through events than the WT mice at three, six, and sixteen months of age. Furthermore, hang-time duration was shorter in *Tnxb* KO mice than in WT mice at all ages; but these differences were not statistically significant (*Table 1*).

Observation during these tests showed that four of the eight *Tnxb* KO mice at 16 months of age hardly moved spontaneously during both trials of the paw-fall-through test. They only slowly moved around in a square of approximately 5 x 5 cm, whereas the WT and other *Tnxb* KO mice moved around the whole mesh hardware cloth (60 x 40 cm). These four KO mice had less than four paw-fall-through events in both trials. Hence, this lack of spontaneous

Figure 1 Results of long term assessment of motor activity showed no statistically significant differences between the wildtype (WT) and tenascin-XB knockout (*Tnxb* KO; KO) mice in hourly walking distance, duration of immobility, duration of mobility, or duration of strong mobility at six or 16 months of age. No statistical significant differences between *Tnxb* KO en WT mice were found.

A and **B:** Graphic representation of hourly distance moved at six and 16 months of age (in cm), with standard errors indicated with error bars. **C**, **D**, and **E:** Graphic representation of hourly duration of immobility, mobility, and strong mobility at 16 months of age (in sec), with standard errors indicated with error bars.

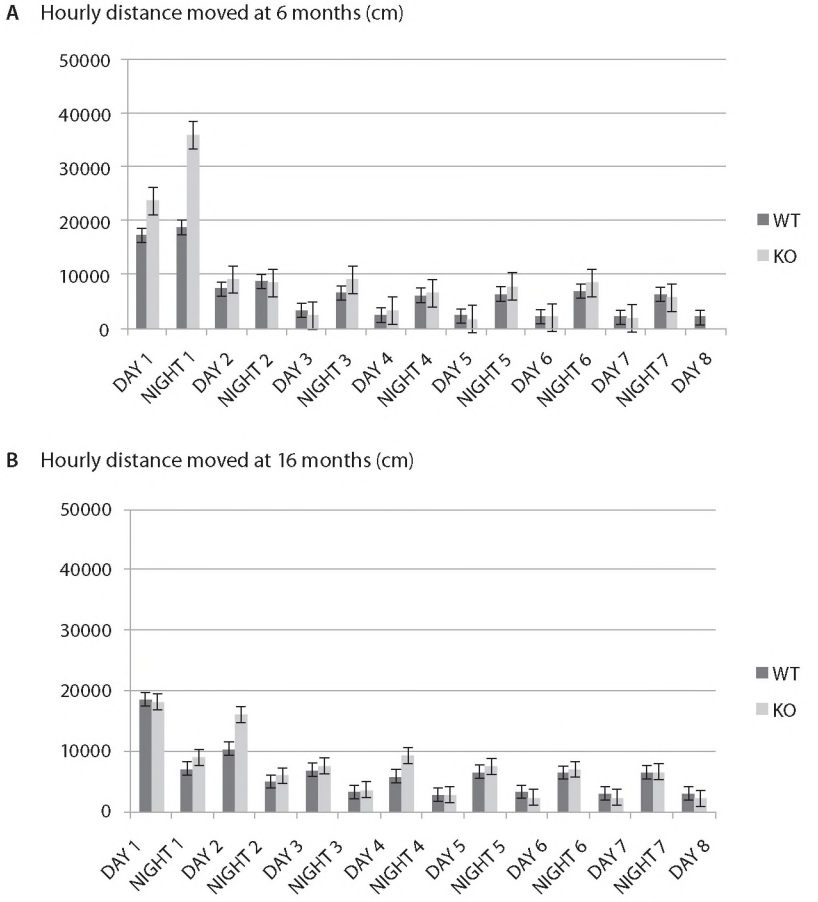
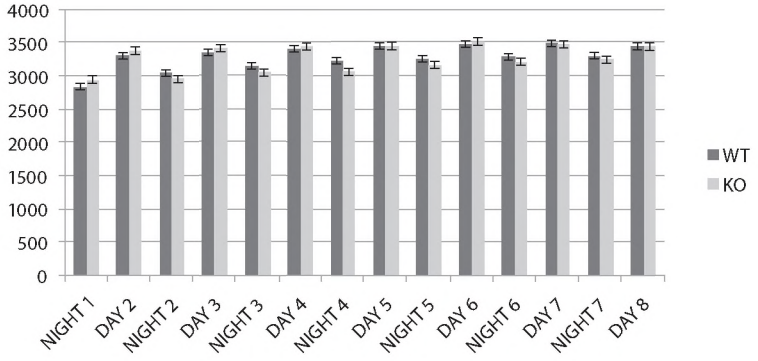
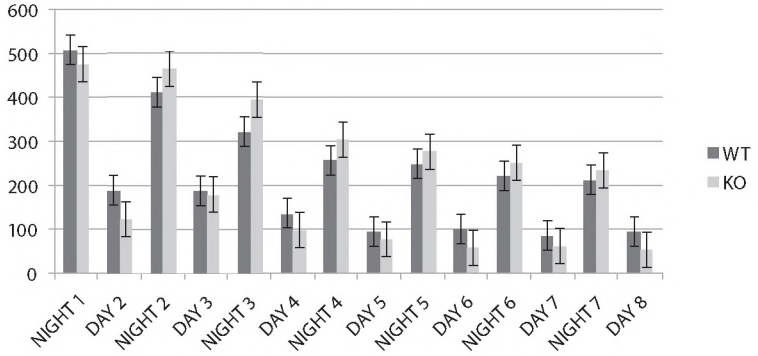


Figure 1 Continued.

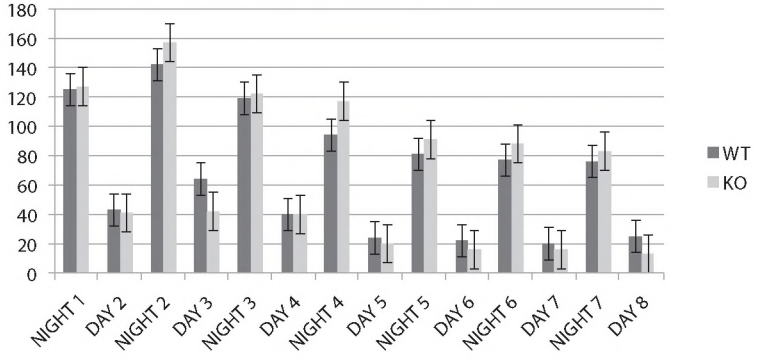
C Hourly duration of immobility at 16 months (sec)



D Hourly duration of mobility at 16 months (sec)



E Hourly duration of strong mobility at 16 months (sec)



movement on the screen in *Tnxb* KO mice might have negatively influenced the results of the paw-fall-through test. Furthermore, results of the hang-time test might have been negatively influenced by the heavier weight of WT mice: at 16 months of age the mean weight was 30.3 g (SD 4.8) in WT mice and 23.1 g (SD 1.5) in the *Tnxb* KO mice (P = 0.03). The two WT mice with a weight > 32 g indeed had a hang-time duration < 120 seconds.

Table 1 Results of hang-time and paw-fall-through test.
Tnxb KO mice showed statistically significantly more paw-fall-through events than the WT mice at three, six, and sixteen months of age. Hang-time duration was shorter in *Tnxb* KO mice than in WT mice at all ages; but these differences were not statistically significant.

	3 months			6 months			16 months		
Number of events / duration	*WT mice n=8*	*Tnxb KO mice n=8*	*WT vs KO P-value*	*WT mice n=8*	*Tnxb KO mice n=8*	*WT vs KO P-value*	*WT mice n=8*	*Tnxb KO mice n=8*	*WT vs KO P-value*
PFT 1 min: mean (SD)	2.3 (1.2)	4.1 (1.0)	0.004#	2.3 (0.7)	3.3 (1.0)	0.041#	3.9 (1.1)	5.1 (1.3)	0.045#
HT dur (ms): median (range)	120 (75-120)	59 (30-120)	n.s.*	120 (51-120)	93 (24-120)	n.s.*	120 (42-120)	93 (34-120)	n.s.*

#: Student's t-test. *: Mann-Whitney-U test. PFT 1 min: number of paw-fall-through events in 1 min. HT dur: hang- hangtime duration. n.s.: not statistically significant.

Quadriceps muscle samples

Histological analysis of quadriceps muscle biopsies of the *Tnxb* KO mice revealed myopathic changes consisting of increase of fibre size variation and increase of internal nuclei (*Figure 2A*). The number of muscle cells with internal nuclei was higher in *Tnxb* KO mice than in WT mice (mean percentage of muscle cells with internal nuclei: 0.76 vs. 5.5; P < 0.001)(*Figure 2B*). Furthermore, Mann-Witney U test in fibre size diameter revealed that the percentage of muscle fibres in the *Tnxb* KO biopsies with a diameter below the p5 of WT mice did not differ from the percentage in WT mice, and that the percentage of muscle fibres with a diameter above p95 of WT mice was higher in *Tnxb* KO mice than in WT mice (P = 0.012)(*Figure 2C*).

For each antibody the sections showed a consistent pattern of staining intensity. Staining of the collagens I, III, and V, elastin, and laminin α2 did not differ between the two groups. Staining of collagen VI was less in *Tnxb* KO mice than in WT mice for perimysial staining (P = 0.003); and endomysial collagen VI staining tended to be less (P = 0.051). The

sections of the *Tnxb* KO mice stained negatively for the TNX-antibody as expected, whereas the WT group showed TNX staining of endo- and perimysium. In general, endomysial staining was less than perimysial staining for collagen I, III, V, and VI in both groups, and for TNX in the WT mice. In contrast, laminin α2 staining was more pronounced in the endomysium than in the perimysium (semi-quantitative data: *Table 2*), compatible with the transmembrane localization of the two major laminin α2 receptors: the dystrophin-glycoprotein complex and integrins (Staining with anti TNX and anti laminin α2 antibodies: *Figure 3*; Staining with antibodies against collagens I, III, and V, VI, elastin, laminin α2, and TNX: *Supplemental figure 1* in original online publication).[29]

Table 2 Semi-quantitative evaluation of immunohistochemical staining of ECM molecules in muscle.
Immunohistochemical staining of the collagens, laminin, and elastin did not differ between the two groups, except collagen VI staining of the perimysium. The sections of the *Tnxb* KO mice stained negative for the TNX-antibody as expected, whereas the WT group showed TNX staining of endo- and perimysium. Endomysial staining was generally less than perimysial staining for collagen I, III, V, VI, and elastin in both groups, and for TNX in the WT mice. In contrast, laminin staining was more pronounced in the endomysium than in the perimysium.

	Collagen I		**Collagen III**		**Collagen V**		**Collagen VI**		**Laminin**		**Elastin**		**Tenascin-X**	
	E	*P*	*E*	*P*	*E*	*P*	*E*	*P*	*E*	*P*	*E*	*P*	*E*	*P*
WT overall score	0.9	2.6	1.6	2.8	2.0	2.6	2.2	3.3	2.6	2.0	0.8	2.4	0.8	1.7
Tnxb KO overall score	1.0	2.7	1.8	2.9	2.3	2.6	1.6	2.3	2.5	2.2	0.7	2.2	0	0
Differences in staining *	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s	0.003	n.s.	n.s.	n.s.	n.s.	P < 0.001	P < 0.001

Mean score of density of staining, based upon semi-quantitative evaluation: 0 = absence; 1 = limited staining; 2 = moderate staining; 3 = considerable staining; 4 = strong staining; E = endomysium; P = perimysium.
*: Mann-Whitney-U test. n.s.: not statistically significant.

Figure 2 Histological analysis of quadriceps muscle biopsies of the *Tnxb* KO mice revealed myopathic changes consisting of increase of fibre size variation and increase of internal nuclei (n = 6 in both groups; of each mouse muscle biopsies were taken bilaterally).

A: HE staining of biopsy of one of the *Tnxb* KO mice (male, eight months), showing myopathic features consisting of increased number of internal nuclei and increased variance of fibre diameter. Bar = 0.05 mm = 50 µm. Normally, nuclei in muscle cells are located peripherally and immediately below the sarcolemma. Internal nuclei are nuclei that are located anywhere else: the arrow points to one of the internal nuclei. This is a selection of an area in which these myopathic features were most pronounced (lower image). In comparison, a normal biopsy of a WT mouse (male, eight months) is presented above. **B:** Percentage of muscle cells with internal nuclei in WT and *Tnxb* KO mice. The outliers are indicated as * and °. The Mann-Whitney U Test was used to test the differences, which showed that internal nuclei were more frequent in *Tnxb* KO mice ($P < 0.001$). **C:** Mann-Witney U test in fibre size diameter revealed that the percentage of muscle fibres in the *Tnxb* KO biopsies with a diameter below the p5 of WT mice did not differ from the percentage in WT mice, and that the percentage of muscle fibres with a diameter above p95 of WT mice was higher in *Tnxb* KO mice than in WT mice ($P = 0.012$).The vertical lines indicate the 5th en 95th percentiles of the diameters in WT mice.

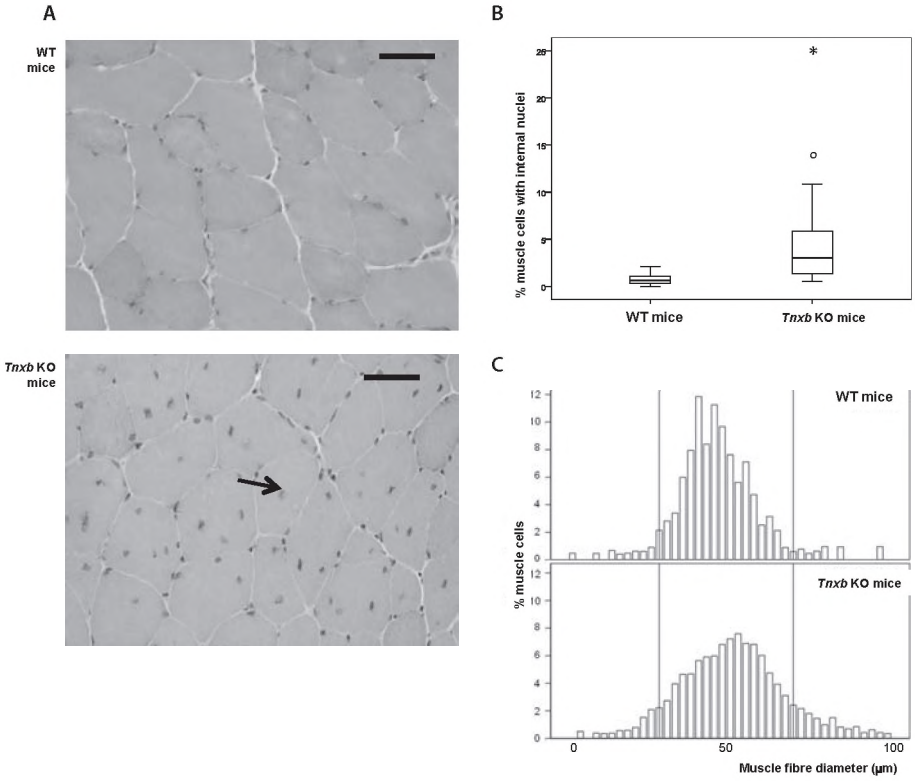
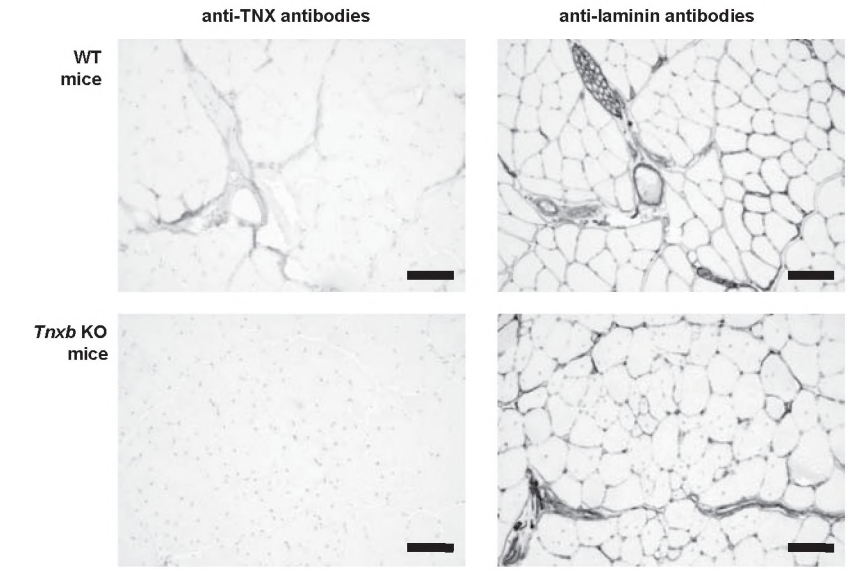


Figure 3 Staining with anti-TNX and anti-laminin α2 antibodies in WT and *Tnxb* KO mice (male, eight months).

Absence of TNX staining in the *Tnxb* KO mice and equal staining of laminin α2 TNX endomysial staining was less pronounced than perimysial staining for TNX in the WT mice, and laminin α2 staining was more pronounced in the endomysium than in the perimysium in both WT and *Tnxb* KO mice. Bar = 0.1 mm = 100 µm.



Gene expression profiling

The muscle function of *Tnxb* KO mice was further studied with genome-wide transcriptome analysis. Total RNA was isolated from quadriceps muscle of 8 months old WT (n = 6) and *Tnxb* KO mice (n = 6) and hybridized to Illumina Mouse Sentrix-6 BeadChips. ANOVA statistical analysis of the microarray data revealed 266 genes differentially expressed ($P < 0.001$, Bonferroni corrected). Of these 74 were downregulated and 192 were upregulated. (*Supplemental table 1* in original online publication).[29] Annotation of the genes with the Webgestalt software enabled the different genes to be classified according to function (*Supplemental table 2 in* original online publication).[29] *Table 3* shows a summary of the functional groups which are overrepresented in the list of differentially expressed genes in *Tnxb* KO mice (Cellular components: lysosome, ECM, and cell surface / Molecular function: carbohydrate binding, peptidase activity, and structural molecule activity. / Biological process: inflammatory response, proteolysis and vascular development). Striking is that the

genes in these functional groups are mainly upregulated. The increased expression of matrix metalloproteinases (MMPs) was reported before in skin of *Tnxb* KO mice[30] and is also observed in the muscles (MMP2, MMP3). The biosemantic analysis showed a list of muscular disorders in which similar genes were shown to be involved in the disease mechanism as in the *Tnxb* KO mice. These neuromuscular disorders are amyotrophic lateral sclerosis, nemaline myopathy, multiminicore myopathy, Charcot-Marie Tooth disease, Duchenne muscular dystrophy, and spinal muscular atrophy.

Table 3 Summary of the functional groups which are overrepresented in the list of differentially expressed genes in TNX KO mice.

Functional group overrepresented	Number of genes	P-value
Cellular component		
Lysosome	10	6.72 E-06
Extracellular matrix	15	5.19 E-05
Cell surface	7	2.93 E-03
Molecular function		
Carbohydrate binding	17	5.96 E-08
Peptidase activity	20	1.42 E-04
Structural molecule activity	14	8.75 E-03
Biological process		
Inflammatory response	15	2.83 E-08
Proteolysis	24	6.83 E-06
Vascular development	8	1.80 E-03

Sciatic nerve sample

Electronmicroscopy of the sciatic nerve revealed many areas with lower density of the connective tissue fibrils in the endoneurium of *Tnxb* KO mouse in comparison with the WT mouse (qualitative evaluation; *Figure 4A*). Furthermore, a few signs of degeneration and regeneration were seen in the sciatic nerve of the *Tnxb* KO mouse. Histometry of the myelinated fibres of the sciatic nerve showed smaller inner and outer diameters in the *Tnxb* KO mouse (Outer diameters: *Figure 4B*). In addition, results of the two-sample Kolmogorov-Smirnov test and Mann-Whitney U test showed that the diameter distributions and central tendencies differed significantly between the *Tnxb* KO mouse and the WT mouse for both inner and outer diameter (*Table 4*).

Table 4 Diameter of myelinated fibres of sciatic nerve in TNX KO and WT mice. Results of the two-sample Kolmogorov-Smirnov test and Mann-Whitney U test showed that the diameter distributions and central tendency differed significantly between the TNX KO mouse and the WT mouse for both inner and outer diameter.

	Diameter of myelinated fibres			
	Myelinated fibres in WT mouse (n=320)	*Myelinated fibres in TNX KO mouse (n=377)*	*Distribution shape difference**	*Central tendency#*
Inner diameter (median (p25 - 75) in µm)	2.3 (1.5-3.6)	2.1 (1.3-3.1)	P = 0.01	P = 0.02
Outer diameter (median (p25 - 75) in µm)	4.2 (2.8-5.8)	3.8 (2.4-5.3)	P = 0.02	P = 0.005

*: Two-sample Kolmogorov-Smirnov test. #: Mann-Whitney U-test.

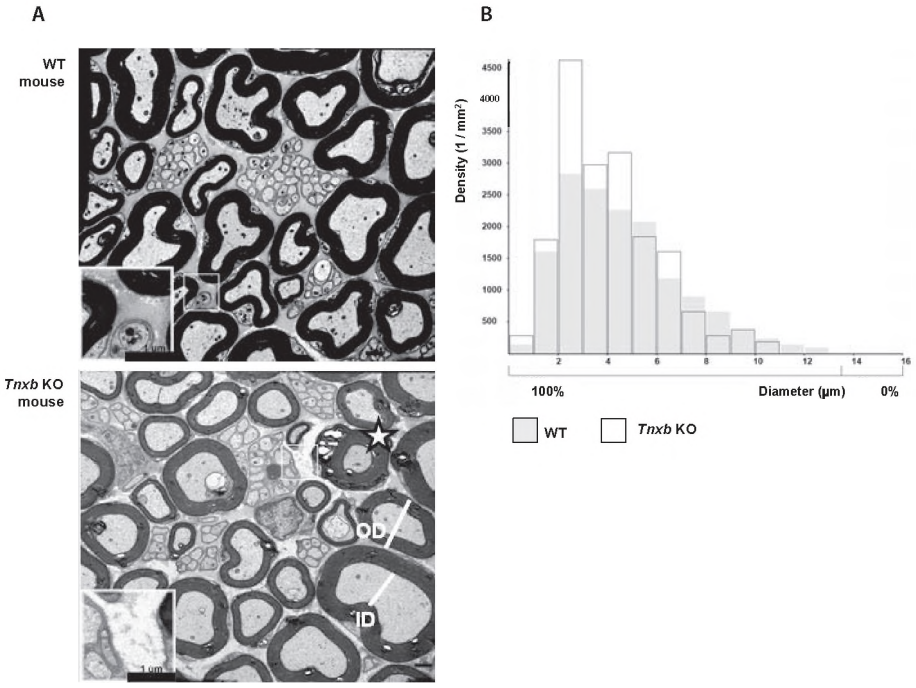
Discussion

The main findings of this study on the muscular phenotype of *Tnxb* KO mice are normal long term spontaneous locomotor activity, presence of mild functional muscle weakness, mild myopathic features on histology, and functional upregulation of genes encoding proteins involved in degradation and synthesis of the ECM in muscle. Additionally, the sciatic nerve specimen showed mildly reduced collagen fibril density of endoneurium between these fibres. Together, these findings point to mild changes in muscle function and composition, and possibly to altered endoneurium composition in *Tnxb* KO mice. Furthermore, the results of gene expression profiling suggest a possible pathophysiological role of TNX deficiency in myopathy in EDS. We will shortly discuss these findings below.

Tnxb KO mice performed less at the paw-fall-through test, whereas differences in performance on the hang-time test were not statistically significant. These differences may have been influenced by the higher weight and longer distances moved in WT mice; a higher weight may predispose to shorter hang-time duration, and the mice that move most on the mesh are at risk for more paw-fall-through events. If we had corrected for these parameters, differences between *Tnxb* KO en WT mice might have been larger. What causes this higher weight in WT mice at 16 months of age has not been investigated further; it might be due to increase of fat, muscle tissue, or body size, or due to water retention or differences in bone mass. Osteoporosis and osteopenia are reported in EDS,[2] and it has been suggested that changes found in skin collagen also occur in bone collagen.[31] Furthermore, the *Tnxb* KO mice tended to move slightly more during the long term spontaneous locomotor activity

Figure 4 Electronmicroscopy of the sciatic nerve in a *Tnxb* KO mouse revealed zones with lower density of the connective tissue fibrils, signs of degeneration and regeneration of myelinated fibres, and smaller inner and outer diameters of myelinated fibres.

A: Electronmicroscopy of the sciatic nerve revealed zones with lower density of the connective tissue fibrils in *Tnxb* KO mice (lower image) in comparison with the WT mice (upper image). The enlarged box in both images magnifies the lower density of connective tissue fibrils in *Tnxb* KO mice. Furthermore, more signs of degeneration (asterisk) and regeneration were seen in the *Tnxb* KO biopsy. Bar = 1 µm. The inner diameter (ID) and outer diameter (OD) of the myelinated fibres have been indicated. **B:** Histogram of outer diameter of myelinated fibres shows a mild shift to the smaller diameters in the *Tnxb* KO mouse. Diameters on x-axis represent the outer diameter of myelinated axons, and the density on the y-axis represents the frequency of the axons of a specific diameter, normalized for the surface measured.



task, but these differences were not statistically significant. Most likely, muscle strength is only mildly reduced in *Tnxb* KO mice, and this reduction does not interfere with spontaneous walking of mice held captive.

Histological analysis of the muscle biopsies revealed mild to moderate myopathic features in the majority of mice; this was confirmed by quantitative analysis of percentage of cells with internal nuclei and of variation of fibre size diameter. This was more pronounced than the myopathic features detected in muscle biopsies of TNX-deficient EDS patients.[20] This might be related to relatively old age of these mice (8 months for mice is late adulthood). The presence of even more pronounced myopathic features we detected in very old *Tnxb* KO mice (22 months; data not shown) supports this. A progression of myopathic features with ageing is also observed in other myopathies and muscular dystrophies.[32,33] Furthermore, no differences in collagen I, III, V, elastin, and laminin α2 staining were found, but perimysial collagen VI staining was less in *Tnxb* KO mice. This is compatible with previous findings of Minamitani et al.[34,35] and our previous observation in a patient with TNX-deficient type EDS.[18]

The results of gene expression profiling a significant upregulation of genes encoding structural ECM components as well as genes involved in synthesis and degradation of the ECM. This probably results from altered interstitial fibroblast function in muscle, which have been shown to contribute significantly to the deposition of the ECM in skeletal muscle.[36] These genes are, according to the available literature, also differentially expressed in various other myopathies such as nemaline myopathy (*TPM2*),[37] and multiminicore myopathy (*SEPN1*).[38] TNX deficiency in perimysium, and to a lesser extent in endomysium may play a role in the development of myopathic features and functional muscle weakness in *Tnxb* KO mice.

The sciatic nerve histology and electromicroscopy revealed mildly reduced diameter of myelinated fibres and reduction of collagen fibril density of endoneurium between these fibres. This might correspond with the presence of mild axonal polyneuropathy in TNX-deficient EDS patients.[20] Clearly, this observation requires further investigation in a larger number of mice. Our findings are in contrast which the results of Matsumoto et al.,[15] who showed that the thickness of myelin sheaths and the size of the individual axons in these mice appeared normal, and that the ultrastructure of the sciatic nerves of *Tnxb* KO mice were similar to those of WT mice. However, quantization of the number and size of sciatic nerve axons from wild-type and *Tnxb* KO mice in this study did show a trend toward smaller axons in *Tnxb* KO mice.[15] The larger differences between *Tnxb* KO and WT mice in our study might be related to the older age of mice.

Tenascin-C (TNC), another member of the tenascin family is predominantly expressed in tendons and ligaments, peripheral and central nervous system, and the ECM of tumour stroma.[39-41] TNC modulates adhesion of cells to fibronectin and can be classified as an adhesion modulating ECM protein. Reduced muscle strength in *Tnc* KO mice has been

described, consisting of reduced grip strength and lower latency to fall on the wire hanging test.[42] Another comparison can be made with lysyl hydroxylase-1 KO mice, a mice model of the kyphoscoliotic type of EDS.[43] These mice show difficulty in locomotion, most likely due to laxity or dislocation of the joints, enhanced by general weakness of the muscles.[43] Neuromuscular function could also be studied in more detail in this mice model.

The results of this current animal study strengthen our recent finding of mild to moderate neuromuscular involvement in patients with various types of EDS.[20] This consisted of axonal sensorimotor polyneuropathy in the TNX-deficient type and mixed myopathic-neurogenic or myopathic features on electromyography in all patients. Hence, with their mild muscular features, the *Tnxb* KO mice form a good fit to the mild neuromuscular phenotype in EDS patients.

Our study has several limitations. First, the use of animals of an advanced age raises doubt whether the abnormalities are of pathological or of biological interest. Furthermore, use of animals of different age and different gender for various investigations may complicate the interpretation of the results. However, due to its explorative design, these results could serve as a starting point for further physiological studies on muscle function in *Tnxb* KO mice.

To summarize, the muscular phenotype of *Tnxb* KO mice consists of mild muscle weakness with histological signs of myopathy and of increased turnover of the ECM in muscle. Furthermore, mildly reduced diameter of myelinated fibres and reduction of collagen fibril density of endoneurium may correspond with polyneuropathy in TNX-deficient EDS patients. Together, these results strengthen the clinical overlap of myopathies and inherited connective tissue disorders caused by ECM defects.[18,25,44,45] They thus support the concept that a normal composition of the ECM is important for adequate functioning of muscle and maybe also of peripheral nerve. Furthermore, the results show that this mouse model can be used for further investigations on the influence of TNX deficiency on muscle and peripheral nerve function. Quantitative muscle function testing of isolated muscles and muscle groups will enable direct measurement of the influence of the ECM alterations on muscle function.[46] Eventually, this may lead to studies on treatment approaches such as training or pharmacological interventions, similarly as in a mouse model of collagen VI myopathies.[47]

Reference List

1. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
2. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
3. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.
4. Zweers MC, Bristow J, Steijlen PM, Dean WB, Hamel BC, Otero M, Kucharekova M, Boezeman JB, Schalkwijk J. Haploinsufficiency of TNXB is associated with hypermobility type of Ehlers-Danlos syndrome. Am J Hum Genet 2003; 73: 214-217.
5. Mao JR, Taylor G, Dean WB, Wagner DR, Afzal V, Lotz JC, Rubin EM, Bristow J. Tenascin-X deficiency mimics Ehlers-Danlos syndrome in mice through alteration of collagen deposition. Nat Genet 2002; 30: 421-425.
6. Bristow J, Carey W, Egging D, Schalkwijk J. Tenascin-X, collagen, elastin, and the Ehlers-Danlos syndrome. Am J Med Genet C Semin Med Genet 2005; 139: 24-30.
7. Egging D, van Vlijmen-Willems I, van Tongeren T., Schalkwijk J, Peeters A. Wound healing in tenascin-X deficient mice suggests that tenascin-X is involved in matrix maturation rather than matrix deposition. Connect Tissue Res 2007; 48: 93-98.
8. Egging D, van den Berkmortel F, Taylor G, Bristow J, Schalkwijk J. Interactions of human tenascin-X domains with dermal extracellular matrix molecules. Arch Dermatol Res 2007; 298: 389-396.
9. Egging DF, van Vlijmen I, Starcher B, Gijsen Y, Zweers MC, Blankevoort L, Bristow J, Schalkwijk J. Dermal connective tissue development in mice: an essential role for tenascin-X. Cell Tissue Res 2006; 323: 465-474.
10. Egging DF, van Vlijmen-Willems I, Choi J, Peeters AC, van Rens D, Veit G, Koch M, Davis EC, Schalkwijk J. Analysis of obstetric complications and uterine connective tissue in tenascin-X-deficient humans and mice. Cell Tissue Res 2008; 332: 523-532.
11. Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. Dev Dyn 1995; 203: 491-504.
12. Matsumoto K, Saga Y, Ikemura T, Sakakura T, Chiquet-Ehrismann R. The distribution of tenascin-X is distinct and often reciprocal to that of tenascin-C. J Cell Biol 1994; 125: 483-493.
13. Zweers MC, Hakim AJ, Grahame R, Schalkwijk J. Joint hypermobility syndromes: the pathophysiologic role of tenascin-X gene defects. Arthritis Rheum 2004; 50: 2742-2749.
14. Geffrotin C, Garrido JJ, Tremet L, Vaiman M. Distinct tissue distribution in pigs of tenascin-X and tenascin-C transcripts. Eur J Biochem 1995; 231: 83-92.
15. Matsumoto K, Sawa H, Sato M, Orba Y, Nagashima K, Ariga H. Distribution of extracellular matrix tenascin-X in sciatic nerves. Acta Neuropathol (Berl) 2002; 104: 448-454.
16. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
17. Behan WM, Longman C, Petty RK, Comeglio P, Child AH, Boxer M, Foskett P, Harriman DG. Muscle fibrillin deficiency in Marfan's syndrome myopathy. J Neurol Neurosurg Psychiatry 2003; 74: 633-638.
18. Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Ehlers-Danlos syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies. Am J Med Genet A 2007; 143: 2215-2219.
19. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
20. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
21. de Visser L, van den Bos R, Spruijt BM. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res 2005; 160: 382-388.

22. van der Kooi AJ, de Voogt WG, Bertini E, Merlini L, Talim FB, Ben YR, Urtziberea A, de Visser M. Cardiac and pulmonary investigations in Bethlem myopathy. Arch Neurol 2006; 63: 1617-1621.
23. Olmstead CE. Neurological and neurobehavioral development of the mutant 'twitcher' mouse. Behav Brain Res 1987; 25: 143-153.
24. van der Laak JA, Dijkman HB, Pahlplatz MM. Automated magnification calibration in transmission electron microscopy using Fourier analysis of replica images. Ultramicroscopy 2006; 106: 255-260.
25. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, Anton-Lamprecht I, Muntoni F, Hanefeld F, Bonnemann CG. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A 2005; 132: 296-301.
26. Turk R, 't Hoen PA, Sterrenburg E, de Menezes RX, de Meijer EJ, Boer JM, van Ommen GJ, den Dunnen JT. Gene expression variation between mouse inbred strains. BMC Genomics 2004; 5: 57.
27. Zhang B, Kirov S, Snoddy J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 2005; 33: W741-W748.
28. Jelier R, Schuemie MJ, Veldhoven A, Dorssers LC, Jenster G, Kors JA. Anni 2.0: a multipurpose text-mining tool for the life sciences. Genome Biol 2008; 9: R96.
29. Voermans NC, Verrijp K, Eshuis L, Balemans MMC, Egging D, Sterrenburg E, van Rooy IALM, van der Laak JWAM, Schalkwijk J, van der Maarel SM, Lammens M, Engelen BG. Mild muscular features in tenascin-X knockout mice, a model of Ehlers-Danlos syndrome. Connect Tissue Res 2011; Mar 15 [Epub ahead of print].
30. Matsumoto K, Minamitani T, Orba Y, Sato M, Sawa H, Ariga H. Induction of matrix metalloproteinase-2 by tenascin-X deficiency is mediated through the c-Jun N-terminal kinase and protein tyrosine kinase phosphorylation pathway. Exp Cell Res 2004; 297: 404-414.
31. Shuster S. Osteoporosis, a unitary hypothesis of collagen loss in skin and bone. Med Hypotheses 2005; 65: 426-432.
32. Dubowitz V, Sewry CA. Muscle Biopsy A practical approach. 2007.
33. Pepe G, de Visser M, Bertini E, Bushby K, Vanegas OC, Chu ML, Lattanzi G, Merlini L, Muntoni F, Urtizberea A. Bethlem myopathy (BETHLEM) 86th ENMC international workshop, 10-11 November 2000, Naarden, The Netherlands. Neuromuscul Disord 2002; 12: 296-305.
34. Minamitani T, Ikuta T, Saito Y, Takebe G, Sato M, Sawa H, Nishimura T, Nakamura F, Takahashi K, Ariga H, Matsumoto K. Modulation of collagen fibrillogenesis by tenascin-X and type VI collagen. Exp Cell Res 2004; 298: 305-315.
35. Minamitani T, Ariga H, Matsumoto K. Deficiency of tenascin-X causes a decrease in the level of expression of type VI collagen. Exp Cell Res 2004; 297: 49-60.
36. Zou Y, Zhang RZ, Sabatelli P, Chu ML, Bonnemann CG. Muscle interstitial fibroblasts are the main source of collagen VI synthesis in skeletal muscle: implications for congenital muscular dystrophy types Ullrich and Bethlem. J Neuropathol Exp Neurol 2008; 67: 144-154.
37. Donner K, Ollikainen M, Ridanpaa M, Christen HJ, Goebel HH, de VM, Pelin K, Wallgren-Pettersson C. Mutations in the beta-tropomyosin (TPM2) gene--a rare cause of nemaline myopathy. Neuromuscul Disord 2002; 12: 151-158.
38. Jungbluth H, Zhou H, Hartley L, Halliger-Keller B, Messina S, Longman C, Brockington M, Robb SA, Straub V, Voit T, Swash M, Ferreiro A, Bydder G, Sewry CA, Muller C, Muntoni F. Minicore myopathy with ophthalmoplegia caused by mutations in the ryanodine receptor type 1 gene. Neurology 2005; 65: 1930-1935.
39. Jones FS, Jones PL. The tenascin family of ECM glycoproteins: structure, function, and regulation during embryonic development and tissue remodeling. Dev Dyn 2000; 218: 235-259.
40. Jones PL, Jones FS. Tenascin-C in development and disease: gene regulation and cell function. Matrix Biol 2000; 19: 581-596.
41. Chiquet-Ehrismann R, Tucker RP. Connective tissues: signalling by tenascins. Int J Biochem Cell Biol 2004; 36: 1085-1089.
42. Morellini F, Schachner M. Enhanced novelty-induced activity, reduced anxiety, delayed resynchronization to daylight reversal and weaker muscle strength in tenascin-C-deficient mice. Eur J Neurosci 2006; 23: 1255-1268.
43. Takaluoma K, Hyry M, Lantto J, Sormunen R, Bank RA, Kivirikko KI, Myllyharju J, Soininen R. Tissue-specific changes in the hydroxylysine content and cross-links of collagens and alterations in fibril morphology in lysyl hydroxylase 1 knock-out mice. J Biol Chem 2007; 282: 6588-6596.

44. Lampe AK, Bushby KM. Collagen VI related muscle disorders. J Med Genet 2005; 42: 673-685.
45. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
46. Rijkelijkhuizen JM, Baan GC, de Haan A, de Ruiter CJ, Huijing PA. Extramuscular myofascial force transmission for in situ rat medial gastrocnemius and plantaris muscles in progressive stages of dissection. J Exp Biol 2005; 208: 129-140.
47. Angelin A, Tiepolo T, Sabatelli P, Grumati P, Bergamin N, Golfieri C, Mattioli E, Gualandi F, Ferlini A, Merlini L, Maraldi NM, Bonaldo P, Bernardi P. Mitochondrial dysfunction in the pathogenesis of Ullrich congenital muscular dystrophy and prospective therapy with cyclosporins. Proc Natl Acad Sci U S A 2007; 104: 991-996.

CHAPTER 9

Muscle characteristics and altered myofascial force transmission in tenascin-X deficient mice, a mouse model of Ehlers-Danlos syndrome

Adapted from:
Huijing PA, Voermans NC, Baan GC, Busé TE, van Engelen BG, de Haan A.
J Appl Physiol. 2010;109:986-95.

Abstract

The Ehlers-Danlos syndrome (EDS) is a group of inherited connective tissue disorders caused by defects in collagens or tenascin-X (TNX). Muscle involvement can be expected based on interactions between muscle and extracellular matrix molecules; however, muscle function has not yet been investigated quantitatively. This study aims to investigate effects of TNX deficiency on muscular characteristics in *Tnxb* knockout (KO) mice, a mouse model of EDS.

This study focused on both intra- and intermuscular aspects of muscle force. Intramuscular aspects were studied during isometric contractions of isolated (maximally dissected) muscles, when the muscle–tendon complex length is fixed. In this situation, the actual active length of the muscle fibres is dependent on the properties of the series elastic components, consisting of the network of endo-, peri-, and epimysium, and of the tendon. Study of the intermuscular aspects of muscle force (in minimally dissected muscles) has proved to be an effective method to investigate myofascial force transmission.

At lower muscle lengths maximally dissected medial gastrocnemius muscle-tendon complex of *Tnxb* KO mice showed lower active force, lower maximal rate of relaxation, and longer time delay between first stimulation pulse and initial force rise, supporting the hypothesis that relatively more slack needs to be taken up as well as more elastic length changes occurs. In addition, study of the minimally dissected lower leg muscles shows that TNX deficiency strongly affects the mechanical interaction between antagonistic as well as synergistic muscles. This is consistent with the concept of altered myofascial force transmission due to increased compliance of myofascial components.

Altered properties of the force transmission pathways of muscle (being either part of the myotendinous or myofascial pathways) due to TNX deficiency directly affect muscle function in *Tnxb* KO mice. In parallel, such effects are likely to contribute to muscle weakness experienced by patients with EDS.

Introduction

The Ehlers-Danlos syndrome (EDS) is a group of inherited connective tissue disorders caused by defects in metabolism of fibrillar collagens. It presents with joint hypermobility, skin hyperextensibility, abnormal scar formation, easy bruising, and tissue fragility.[1,2] EDS is caused by mutations in the genes encoding collagen I, III and V, and tenascin-X (TNX), molecules which are known to be abundantly expressed in the extracellular matrix (ECM).[1-3]

Primary muscle involvement in EDS can be expected based on interactions between muscle and these ECM molecules.[4] In a recent case study we indeed demonstrated reduced muscle function in two EDS patients, which could not be attributed to increased tendon compliance or disuse.[5] Subsequently, we found considerable clinical muscle weakness in patients with various types of EDS accompanied by mild histological myopathic changes.[6]

This study aims to investigate the effects of TNX deficiency on muscular characteristics in *Tnxb* knockout (KO) mice, a mouse model of EDS. Various intra- and intermuscular aspects of muscle force may be affected by TNX deficiency. Intramuscular aspects can be studied during isometric contractions of isolated muscles. During an isometric contraction the muscle–tendon complex length is fixed, but the actual active length of the muscle fibres is dependent on the properties of the series elastic components, consisting of the network of endo-, peri-, and epimysium, as well as of tendon. Similarly, these properties affect the rate of length change of the fibres during the initial phase of force generation, and hence influence the rate of force building up. These intramuscular aspects of muscle force may be affected by TNX deficiency via altered visco- elastic properties of the connective tissue within muscle and tendon.

Study of the intermuscular aspects of muscle force has proved to be an effective method to investigate myofascial force transmission.[7] This concept is based on the ability of muscle to transmit forces between muscle fibres and connective tissue within muscle (endo- and perimysium) and between individual muscles and connective tissue between muscles (epimysium, fascia, septum, neurovascular tract). As a result, morphologically defined muscles are not independent actuators, but are capable of mechanical interaction via their connective tissue structures.[8-10] As such, force exerted at the origin of a muscle within its natural context of connective tissue is not necessary equal to the force exerted at its insertion, since additional loads initiated in neighbouring muscles act on the muscle. Hence, the difference in forces measured at the muscle's origin and insertion is an unequivocal indication for net epimuscular myofascial force transmission.[9] Furthermore, as epimuscular myofascial force transmission is mediated by surrounding connective tissues, the fraction of force transmitted myofascially has been found to depend on muscle length and its position relative to its surrounding structures.[11]

In view of the above, we hypothesize that TNX deficiency affects not only intramuscular aspects of muscle force via altered visco-elastic properties of the connective tissues; it also

reduces the stiffness of myofascial pathways, causing pathological changes in force transmitted this way. The current study therefore aims to investigate directly the effect of TNX deficiency on muscle characteristics in a mouse model of TNX-deficient type EDS.[12] To do so, this study combines measurement of both intramuscular and tendon aspects (i.e. force characteristics of the maximally dissected medial gastrocnemius muscle tested in isolation; *series A*) and intermuscular aspects (i.e. force characteristics of the triceps surae muscle and anterior crural muscles without major dissection to detect changes in mechanical interaction between these muscle groups; *series B*). Contractile responses from *Tnxb* KO mice will be compared with those from wild-type (WT) mice.

Material and methods

The experimental design was approved by the Ethics Committee for Animal Experimentation of the Vrije Universiteit Amsterdam.

Tenascin-XB knockout mice

Tnxb KO mice were obtained as previously reported by inactivating murine *Tnxb*.[12,13] The 5' end of the gene was targeted, thus replacing the first five coding exons with lacZ and a neomycin resistance cassette. Correct targeting of Tnxb was confirmed by Southern blotting and, as expected, *Tnxb* KO mice lacked both TNX mRNA and protein.[12] Experiments were performed on two groups of *Tnxb* KO mice that had been crossed back with six generations of C57BL/6N mice.

Series A: Eight female *Tnxb* KO mice (mean body mass of 39.3 g., SE = 0.6 g.) and seven female WT C57BL/6 (WT; mean body mass of 41.6 g., SE = 1.5 g.) mice were tested (age 12 - 14 months).
Series B: Six female *Tnxb* KO mice (mean body mass of 30.2 g., SE = 0.91g.) and six female WT C57BL/6 (WT) mice were used as a control (mean body mass 31.2 g., SE = 2.49 g.) (age 12 - 14 months).

All mice were deeply anaesthetized by administration (i.p. 0,1 ml /10g g body mass) of a solution of fentanyl citrate (0,079 mg/ml) and fluonizole (2.5mg/ml) (Hypnorm®) and midazolam (1.25 g/ml)(Dormicum®). Additional doses of were given as necessary (0.05 ml or 0.10 ml, i.p.). During surgery and data collection, animals were placed on a heated water pad of approximately 37 °C to prevent hypothermia.

Table 1 Biomechanical concepts in this study.

Concept	Abbreviation (unit)	Description
Optimum length	ℓ_o (mm)	The muscle's length at which the length of the muscle's sarcomeres are, on average, on the plateau of the length-force curve; this represents the length at which actin and myosin have a maximal overlap.
Active slack length		Active slack length is the lowest muscle length at which active force approaches zero. At any length below that length the active muscle is slack. Below active slack length even a fully active muscle does not exert force on its outside world, and the distance between its proximal and distal end may not adequately reflect its true length due to buckling of tissues.
Muscle slack(ness)		A characteristic of the series elastic components of muscle; it reflects the necessity to stretch the series elastic components minimally before they can transmit forces; this is referred to when it is mentioned that a muscle needs time to take up slackness before shortening once contraction has started.
Passive force	Fmp (mN)	The force required to stretch a relaxed muscle to a given length.
Active muscle force	Fma (mN)	Total force minus passive force; this estimates the component of force that is related to the attachment of cross bridges.
Normalized active force	%Fma	Active force at a given length as a percentage of active force at optimum length (ℓ_o).
Total force	Fmt (mN)	The final force that a muscle attains following stimulation. This force includes the passive force that existed prior to stimulation and the component of force that is generated in response to the stimulus.
Optimum force	F_o (mN)	Maximal active muscle force (at ℓ_o) in a length-force curve.
Active peak force	peakFma (mN)	Maximal active muscle force in a force-time curve for each given length; these peak forces at a certain length are plotted in the length-force curve. The active peak force at ℓ_o equals the optimum force.
Normalized maximal rate of relaxation	%MRR (mN/ms)	Maximal rate of relaxation as a percentage of the rate of relaxation after the first contraction (in isometric fatigue protocol); after stimulation with 150 Hz (in frequency-force measurements); or after contraction at ℓ_o (in length-force measurements).
Normalized maximal rate of force rise	%MRFR	Maximal rate of force rise as a percentage of the rate of force rise after the first contraction (in isometric fatigue protocol); after stimulation with 400 Hz (in frequency-force measurements); or after contraction at ℓ_o (in length-force measurements) (mN/ms).
Maximal power production	(mW)	Highest power (muscle force x velocity) obtained from the fitted power - velocity curve.

Surgical procedures

Dissection of sciatic nerve

The sciatic nerve was dissected free from surrounding tissues and severed as proximally as possible. Subsequently, all of its branches except the branch to the medial gastrocnemius (*series A*) or the common peroneal and tibial nerves (*series B*) were cut. To be able to clamp the femur, small insertions were made in the musculature located anteriorly and posteriorly of the femur, and a metal clamp was inserted and tightened.

Dissection of muscles

Series A: Maximally dissected medial gastrocnemius muscle

The medial gastrocnemius muscle (GM) was fully dissected from the surrounding tissue, with the exception of blood supply and innervating nerve.

Series B: Anterior crural and Triceps surae muscles

For this segment of the experiments, dissection of the lower leg was minimized to free the distal tendons of target muscles. Only limited fasciotomy was performed distally to expose the distal tendons of the tibialis anterior muscle (TA), extensor hallucis longus muscle (EHL) and extensor digitorum longus muscle (EDL), and to sever the retinaculae (i.e. the transverse crural ligament and crural cruciate ligament). Otherwise the connective tissue at the muscle bellies and tendons was left intact. The distal tendons of EDL were tied together (Ethilon surgical suture) and severed distally of the knot. Also, the distal tendons of TA and EHL were tied (polyester yarn) and severed from their insertions. Below, this complex will be referred to as TA+EHL complex. A small piece of the epicondylus lateralis comprising the origin of the EDL muscle was cut from the femur. Similarly, a small piece of bone was cut, comprising the insertion of the triceps surae muscle (TS) via the Achilles tendon on the calcaneus bone. All tendons described were tied to kevlar threads (4% elongation at a break load of 800 N), that were in turn attached to rods for later connection to a force transducer. In the reference position (corresponding to a knee angle of 100° and ankle angle of 180° plantar flexion), the original position of the proximal tendon of EDL on the epicondylus lateralis of the femur was marked by placing corresponding markers on the proximal EDL tendon and lateral collateral ligament. The foot was firmly attached to a plastic foot plate (*Figure 1A and 1B*).

Experimental set-up, conditions, and treatment of data

Series A: Maximally dissected medial gastrocnemius muscle

The distal tendon of the GM (length ~ 4mm) was connected to a force transducer of an isovelocity measuring system.[14] The attachment of the proximal tendon was left intact. The femur was fixed to the measuring system. Length changes of the GM tendon complex were induced with a computer-controlled servomotor connected to a lever on which the force

transducer was mounted. Contractions were induced by electrical stimulation using a constant current stimulator. Electrical pulses (width 50 μs) were applied to the sciatic nerve with a constant current (1 mA), being high enough to fully activate all muscle fibres. The muscle temperature was maintained at 34 – 36 °C with a water-saturated airflow around the muscle, which at the same time kept the muscle moistened.[14] Force and length signals were digitized (1 - 5 kHz) and stored on disc. At the end of the experiment, the medial gastrocnemius muscles were excised and weighed. Thereafter, the mice were humanely killed with an overdose of anaesthesia.

Muscle optimum length was first estimated using a few twitch contractions (one per minute). (Tetanic) optimum length (ℓ_o) was subsequently determined using only 3 – 4 tetanic contractions (stimulation frequency 150 Hz, duration 150 ms). About 10 min later, the following series of contractions started. Duration of a single pulse in all experiments was 50 μs.

Length-Force protocol

Muscles were stimulated isometrically in random order at various lengths (steps of 0.5 mm) between ℓ_o-4 and ℓ_o+2 mm for 150 ms with a stimulation frequency of 150 Hz with >2 min rest intervals to prevent fatigue.

Stimulation frequency

Contractions were performed at ℓ_o using the following stimulation frequencies 25, 50, 75, 100, 250 and 400 Hz and pulse duration of 50 μs with durations long enough to allow the muscles to reach their peak force at each particular frequency. Between the contractions there was at least 2 min rest.

Force-Velocity protocol

Contractions were performed during which the muscles were allowed to shorten at different constant velocities (at random; 0, 20, 30, 40, 50, 75, and 100 mm/s). Just before the start of the contraction, the muscle was (passively) stretched to 0.5 mm over ℓ_o. During the initial part of the contraction, the length of the muscle was kept constant until the (increasing) force had reached the level that was estimated to be the force that could be sustained during the shortening at the imposed velocity. In this way, the measured force was constant when the muscle passed ℓ_o during shortening.[15]

Hence, the velocity of the length change of the muscle tendon complex is also the velocity of shortening of the muscle fibres, since no length change occurs in the series elastic elements at constant force. The stimulation frequency used was 400 Hz, except for contractions with shortening velocities of 0 and 20 mm/s, where 200 Hz was used. These frequencies were high enough to obtain maximal forces at all shortening velocities. After each contraction, there was at least 2 min rest.

Fatigability protocol

A series of 20 repeated isometric contractions was induced at ℓ_o (duration 150 ms, one contraction every 500 ms, and stimulation frequency 150 Hz).

Data management

From the isometric force traces the following parameters were calculated: Active peak force (peak Fma; mN) was taken as the highest force minus the passive force. The maximal rates for force rise (%MRFR) and relaxation (%MRR in mN/ms) were taken as the maxima and minima of the differentiated force signal at the beginning and end of the contraction, respectively. The above data were normalized for the data obtained 1) at ℓ_o to study the influence of length, 2) at a stimulation frequency of 250 Hz (for Fma) and 400Hz (for MRFR and MRR) to study the influence of stimulation frequency and 3) to the first contraction of the series to study the fatigability and all data are presented as percentage. The time (ms) between the first stimulation pulse and the increase of force above 2% of the maximal force was determined as time needed to take up the slack of the muscle. For the shortening contractions, the force was obtained when the muscle passed ℓ_o. Power was calculated by multiplying the force by the imposed velocity (mW). For each muscle maximal power was obtained from the fitted curve through the power-velocity data points.

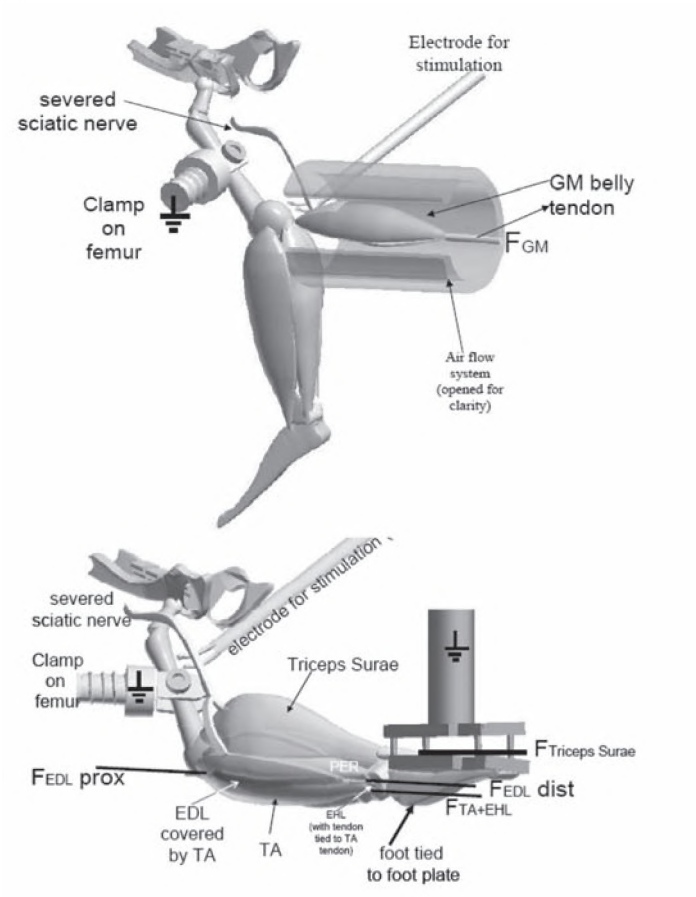
Series B: Anterior crural and Triceps surae muscles

The animal was mounted in the experimental set-up, at a knee angle of approximately 110 °, (measured post-experimentally in images to be equal to mean ± SE 111.8 ° ± 1.4 ° and 111.8 ± 5.1 ° for the *Tnxb* KO and WT group respectively). The foot, attached to a plastic plate, was attached to a rigid frame with the ankle in extreme plantar flexion to create room for free passage of the distal tendons of EDL and TA+EHL at the ankle. The distal tendons of TA+EHL and EDL as well as the proximal EDL tendon were connected to force transducers (ME-Meßsysteme GmbH, Germany, compliance of 0.025 mm/N) mounted on single-axis micro-positioners. Also the kevlar thread attached to TS distal tendons was attached to a force transducer. The sciatic nerve was placed on a pair of silver electrodes and prevented from dehydration by covering it with paper tissue saturated with isotonic saline and a thin piece of latex.

Ambient temperature (22 ± 0.5 °C) and air humidity (70 ± 2%) were kept constant by a computer-controlled air-conditioning system (Holland Heating, Waalwijk, the Netherlands). Muscle and tendon tissue was further prevented from dehydration by regular irrigation with isotonic saline. Before acquiring length-force data, EDL was preconditioned by isometric contractions at alternating high (ℓ_o) and low (ℓ_o - 3) lengths, until active forces at low length were reproducible (i.e. effects of previous activity at high length are minimized).[16] The proximal EDL tendon was set at a position 1 mm distal of the marker position on the femur (i.e. shorter muscle). Throughout the experiment, the proximal tendon of EDL was kept at this position. The EDL distal tendon was set at 1 mm below its optimum length and kept at that position during the experiment. Also for TS, as well as TA+EHL temporary estimates of optimum force (i.e. the highest active force measured as a function of length) and optimum

Figure 1 Experimental set-up of the experiments.

A: Experimental set-up of *series A*: Maximally dissected medial gastrocnemius (GM):
The sciatic nerve was dissected free from surrounding tissues and severed as proximally as possible. Subsequently, all of its branches except the branch to the GM were cut. To be able to clamp the femur, small insertions were made in the musculature located anteriorly and posteriorly of the femur, and a metal clamp was inserted and tightened. Subsequently, the GM was fully dissected from the surrounding tissue, with the exception of the blood supply and the innervating nerve.
B: Experimental set-up of *series B*: Minimally dissected Anterior crural and Triceps surae muscles: a limited fasciotomy was performed distally to expose the distal tendons of the tibialis anterior muscle (TA), extensor hallucis longus muscle (EHL) and extensor digitorum longus muscle (EDL), and to severe the retinaculae. The distal tendons of EDL were tied together and severed distally of the knot. Also, the distal tendons of TA and EHL were tied. (TA+EHL complex), severed from their insertions and attached to the force transducer. Also the proximal EDL tendon was attached to a force transducer. The lines of pull of EDL, TA+EHL and TS muscles were aligned with the line of pull of their respective force transducers. The sciatic nerve was placed on a pair of silver electrodes was to activate the muscles of the lower leg.



length (the length of occurrence of the highest active force) were estimated. These values were used only during the execution of the experiment.

Length-force protocol

During measurement of TS length-force characteristics, muscle-tendon complex length of TA+EHL complex was kept relatively short (i.e. on the ascending limb of its length force curve). Initially this length corresponded to an active force of approximately 1/3 of optimum force (1/3 Fmao). Similarly, for measurement of the TA+EHL length-force characteristics, TS muscle-tendon complex length was not changed and kept at a length initially corresponding to an active force of approximately 1/3 of optimum force. Therefore, if no myofascial muscular interaction would occur one would expect TA+EHL and TS muscle forces respectively to remain constant during measurements of length-force characteristics of its antagonistic muscle group.

Prior to excitation of the sciatic nerve, all muscles were brought passively to the desired lengths by moving the distal positioners (Muscle to be manipulated; stepwise per 0.5 mm; other muscles at length corresponding to that yielding an active force of approximately 1/3 of optimum force.) The imposed length change was read from the micromanipulator to the nearest 0.1 mm. Post-experimentally changes in muscle-tendon complex length are expressed as deviation from optimum length. All muscles were activated simultaneously by supramaximal stimulation of the sciatic nerve with a constant current (< 3 mA) and a stimulation frequency of 100 Hz (pulse width 0.5 ms). Two twitches were evoked, followed by a tetanic contraction of 300 ms. For a typical example of force data collected see *Figure 2*. Timing of stimulation and analog-to-digital (A/D) conversion of force data (12-bit A/D converter, sampling frequency 1000 Hz) was controlled by a special purpose microcomputer. After each tetanic contraction, the muscles were allowed to recover near active slack length for 2 minutes. Passive isometric force was measured prior to the tetanic contraction and total force was measured at a point during the final quarter of the tetanic force plateau.

Treatment of data

Passive muscle force (Fmp), as a function of muscle tendon complex length, was fitted with an exponential curve using a least-squares criterion:

$$y = \exp(ax + b) + C,$$

where y represents passive muscle force, x represents muscle-tendon complex length and a, b and C are fitting constants. Active muscle force (Fma) was estimated by subtracting from total force (Fm) actually measured the passive force (Fmp) for the appropriate muscle length, calculated using the fitted exponential function. Active length-force data thus obtained were then least square fitted applying a stepwise polynomial regression procedure (see section on statistics):

$$y = b0 + b1x + b2x^2 + \ldots. bnx^n,$$

where y represents active muscle force, x represents active muscle length and b0 through bn are fitting constants. For TS, as well as TA+EHL, optimum length of the muscle-tendon complex (ℓ_o) to be used in further analysis was defined, for each individual muscle, as the muscle length at which the fitted active force curve showed a maximum. TS and TA+EHL distal active slack lengths were estimated by selecting data at lower muscle lengths (Fma < 0.3 x Fmao) and extrapolated using the fitted curve;

$$y = \exp(b0\ x + b1) + b2\ ,$$

where y represents muscle active force, x represents active muscle force length and b0 through b2 are fitting constants.

Similar polynomial fitting procedures were applied for forces exerted in the active and passive states by EDL at its proximal and distal tendons, as well as TS and TA+EHL distal forces in the case where they were not lengthened. For EDL, the differences in passive and active force exerted at the distal and proximal tendon (ΔFmp EDL(Fdist - Fprox) and ΔFma EDL(Fdist - Fprox) respectively) were calculated by subtracting proximal from distal force as determined from the polynomials. For all forces studied and with use of the selected polynomials, mean and standard errors of active and passive muscle force were calculated. This was done so for given deviations from respective optimum lengths of muscles that had been changed in muscle - tendon complex length to measure it length-force characteristics.

Statistics

Series A: Maximally dissected medial gastrocnemius muscle

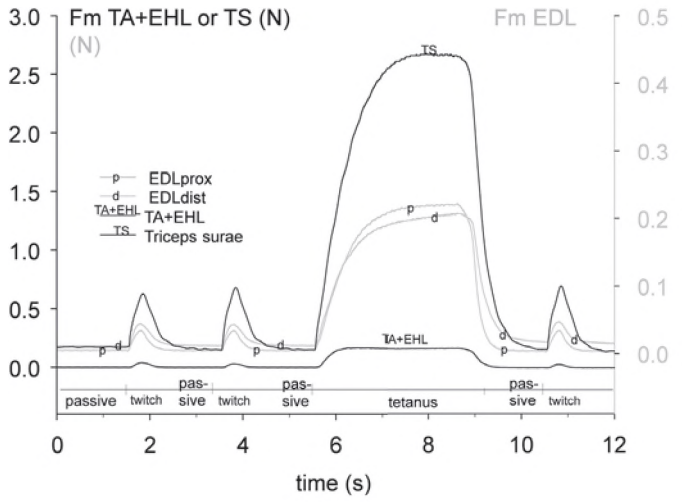
Possible differences in GM maximal isometric force, muscle mass and peak power between the *Tnxb* KO and the WT group were determined using the Student *t*-test. A repeated-measures analysis of variance was used to determine the differences in the effects of stimulation frequency, length and fatigue between the groups. The significance level was set at 0.05.

Series B: Anterior crural and Triceps surae muscles

For curve fitting of TS and TA+EHL active length-force data, the procedure starts with a first order polynomial and the power was increased up to the sixth order, as long as this yields a significant improvement of the statistical description of the length-active force data, as determined by one-way analysis of variance (ANOVA).[17] Bivariate analyses of variance (SPSS version 14.0) were used to test for significance of main effects of muscle – tendon complex length (repeated measurements) of both TS and TA+EHL, of the presence of TNX deficiency, and of their interaction. This was done for: 1) TS active and passive length force characteristics; 2) TA+EHL active and passive length force characteristics; 3) distally exerted EDL active and passive forces; 4) proximally exerted EDL active and passive forces; and on 5) active and passive EDL proximo-distal EDL force differences. Additionally, for lengthening of TS, analyses of variance were used to determine effects of TS length: 6) on active and passive force

Figure 2 A typical example of raw force data collected as a function of time.

Force at exerted at its distal tendon by the triceps surae complex (TS). Force exerted at the tied distal tendons of m. tibialis anterior and m. extensor hallucis longus (TA+EHL). Forces exerted by m. extensor digitorum longus at its proximal tendon (EDL prox) and at its tied distal tendons (EDL dist). Note differences between these two force tracings that indicate epimuscular myofascial force transmission between EDL and its surroundings. For TS and TA+EHL forces refer to left y–axis (drawn in black) and for EDL force to the right y-axis (drawn in grey). All muscles were activated maximally with all motor units recruited. Approximate timing of stimulation is provided on the line inserted just above the x-axis.



exerted distally by TA+EHL while kept at unchanged relatively short muscle tendon complex length; and for lengthening of TA+EHL; and 7) on active and passive force exerted distally by TS, while kept unchanged relatively short muscle tendon complex length. In addition, t-tests were used to test for differences in distal active slack length of TS and TA+EHL.

Results

Series A: Maximally dissected medial gastrocnemius muscle

GM Muscle length

Maximal forces were not different between WT (1.74 ± 0.38 N) and *Tnxb* KO mice (1.47 ± 0.36 N). Actual GM muscle belly length (excluding distal tendon length) measured at optimum

length was not different between the groups (12.85 ± 5.12 mm vs. 13.14 ± 0.69 mm for *Tnxb* KO and WT, respectively). Only at low lengths normalized active isometric force (%Fma) was significantly lower in *Tnxb* KO mice compared to WT mice (at ℓ_o – 4, ℓ_o – 3.5, and ℓ_o – 3; P = 0.030, P = 0.026, and P = 0.032 respectively) (*Figure 3A*). At these low muscle lengths, the relaxation was slower in *Tnxb* KO mice as indicated by a significantly lower maximal rate of relaxation (%MRR) (at ℓ_o – 4, ℓ_o – 3.5, and ℓ_o – 3; P = 0.026, P = 0.022, and P = 0.038 respectively) (*Figure 3B*). Note that although these differences seem small, the relative effects are substantial. Force and relaxation rate in *Tnxb* KO mice were ~ 65% of WT at ℓ_o - 3mm and ~ 46% at ℓ_o - 3.5mm. Furthermore, the delay between the first stimulus pulse and the increase of force above 2% of the maximal active force (2%Fact) was significantly longer in *Tnxb* KO mice at short length (ℓ_o – 4 and ℓ_o – 3.5; P = 0.025 and P = 0.026 respectively)(*Figure 3C*), indicating that relatively more slack is needed to be taken up at shorter lengths in *Tnxb* KO mice.

GM stimulation frequency

There were no significant differences between *Tnxb* KO mice and WT mice in isometric peak forces, normalized maximal rates of force rise, and normalized maximal rates of relaxation at any stimulation frequency applied at ℓ_o or at ℓ_o – 3.5 (data not shown).

GM Shortening velocity

No significant differences in force-velocity characteristics and maximal power production were found between *Tnxb* KO (18.2 ± 4.3 mW) and WT mice (20.9 ± 6.3 mW).

GM Isometric fatigue

The fatigue protocol lead to a similar reduction in force in *Tnxb* KO mice and WT mice (73.2 ± 4.0% and 70.4 ± 5.1% respectively). There were no significant different changes in the maximal rate of force rise and maximal rate of relaxation between *Tnxb* KO mice and WT mice during the series of repeated isometric contractions.

Figure 3 Effects of changes in muscle length on isometric force, maximal rate of relaxation and delay between stimulation and force increase in WT and *Tnxb* KO mice (*Tnxb* -KO).

Muscle length is expressed as deviation from optimum length (ℓ_o). **A:** Normalized active isometric force is significantly lower at low lengths (ℓ_o– 4 mm, ℓ_o– 3.5 mm, and ℓ_o– 3 mm) in *Tnxb* KO mice compared to WT mice. **B:** Significantly lower maximal rate of relaxation at these low muscle lengths (ℓ_o– 4, ℓ_o– 3.5, and ℓ_o– 3 mm) **C:** Significantly longer delay between the first stimulus pulse and the increase of force above 2% of the maximal active force in *Tnxb* KO mice at short length (ℓ_o– 4 mm and ℓ_o– 3.5 mm).

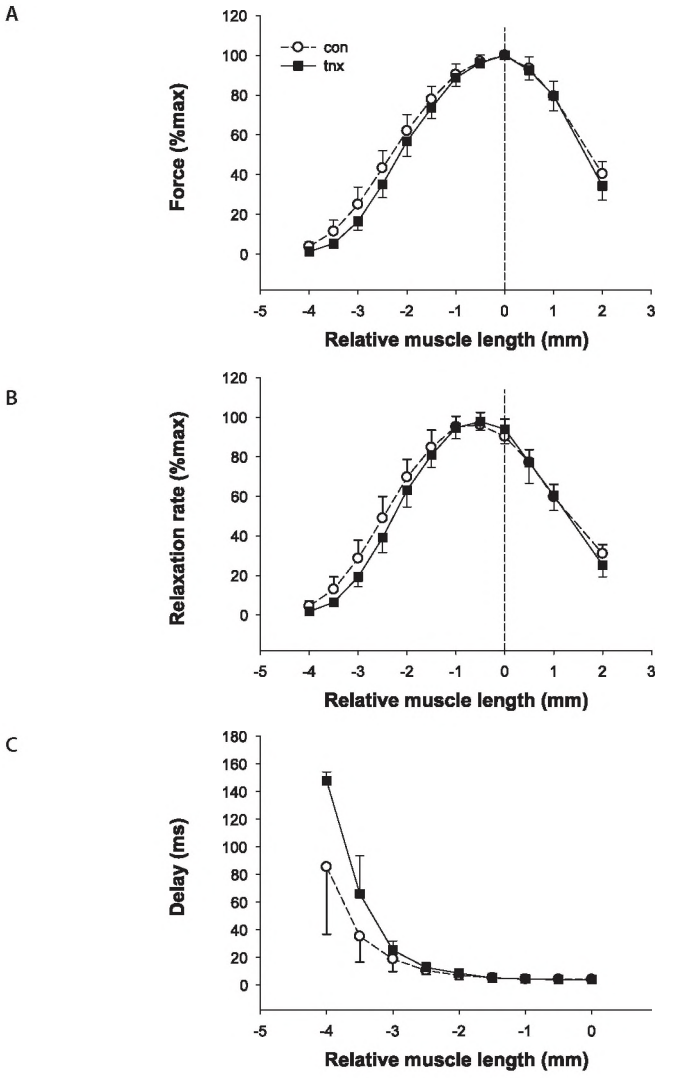
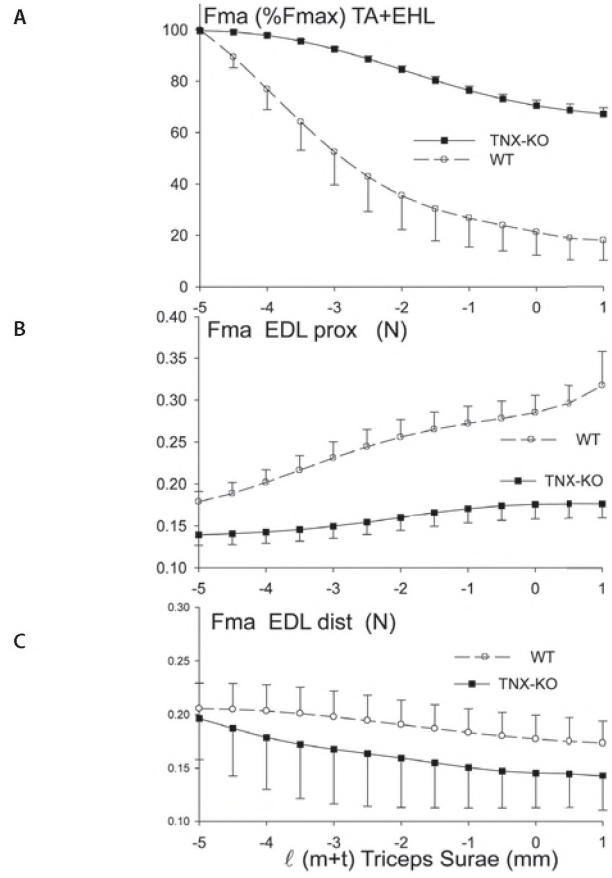


Figure 4 Effect of changes in m. triceps surae length on forces exerted by antagonistic muscles kept at constant length.

A: Active force (Fma) exerted by the m. tibialis anterior-m.extensor hallucis longus complex (TA+EHL) with changing lengths of TS: Normalized active TA+EHL force decreased substantially (by max. 80% of initial force) as a function of increasing TS length. The effect of TNX deficiency is to limit this TS length dependent decrease in normalized active force to levels not exceeding 33% of initial force. **B:** Proximal EDL active force (Fma prox) exerted by m. extensor digitorum longus (EDL) with changing lengths of TS: Proximal active force increases (max. increase > 0.1 N) as a function of increasing TS length. This effect is still present but less pronounced in *Tnxb* KO mice. **C:** Distal active force (Fma dist) exerted by EDL with changing lengths of TS: Distal EDL active force decreases as a function of increasing TS length. This effect is still present but less pronounced in *Tnxb* KO mice. Δl(m+t) is the symbol used to indicate change of muscle tendon complex length expressed with respect to optimum length.



Series B: Anterior crural and triceps surae muscle forces

Effects of TS length change on forces exerted by antagonistic muscles at constant length

(a) Effects on TA+EHL

ANOVA showed significant effects of TS length on active forces exerted by antagonistic muscles, while the length this complex (TA+EHL) was kept unchanged and relatively short. Normalized active force decreased substantially (by max. 80% of initial force) as a function of increasing TS length (*Figure 4A*). ANOVA also indicated significant effects of TNX deficiency, but no significant interaction could be shown. The effect of TNX deficiency is to limit this TS length dependent decrease in active force to levels not exceeding 33% of initial force.

(b) Effects on EDL

Proximal force

ANOVA showed significant main effects (i.e. TS length, TNX deficiency) on EDL proximal active force, as well as significant interaction. Note that proximal active force increases (max. increase > 0.1 N) as a function of increasing TS length. These effects are still present but less pronounced in *Tnxb* KO mice (*Figure 4B*).

Distal force

ANOVA showed significant effect of TS length on EDL distal active force, as well as significant interaction of effects of TS length and TNX deficiency. Note that distal active force decreases as a function of increasing TS length (*Figure 4C*).

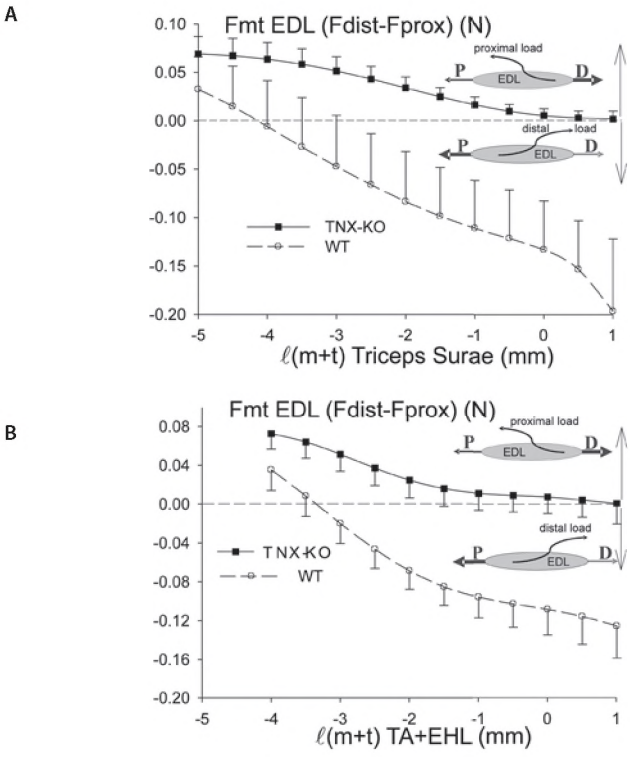
Proximo-distal EDL total force differences

This difference in force exerted in the proximal and distal tendons of EDL is indicative of net epimuscular myofascial force transmission between EDL and other muscular or non-muscular tissues. A positive difference (distal force exceeding proximal force), indicates that a net load is exerted on EDL in proximal direction. The force corresponding to this load is integrated into the force exerted in the distal tendon (see schematics inserted into *Figure 5*). For negative proximo-distal forces differences the reverse is the case. ANOVA showed significant effects of TS length on the EDL total proximo-distal force difference, as well as significant interaction of effects of TS length and TNX deficiency. Therefore, it is concluded that both TS length and TNX deficiency affect myofascial force transmission within the mouse lower leg (*Figure 5A*). At very low lengths (i.e. for TS ℓ_o < -4) a proximal load is exerted on EDL in both WT and *Tnxb* KO mice. The pattern of change of this difference varies substantially between WT and *Tnxb* KO mice: with increasing TS length in WT mice, the EDL force difference decreases rapidly to change sign (i.e. load direction, at approximately TS ℓ_o < -4) and this distally directed load increases substantially with further increasing TS length. For *Tnxb* KO mice, the proximo-distal EDL force difference deceases gradually (to $\Delta F = 0$ at high TS length), thus not changing direction at all. It is concluded that TNX deficiency significantly affects myofascial force transmission, not only in the magnitude of the net myofascial load on EDL, but also with regard to the direction of loading (*Figure 5*). Comparison with *Figure 4B* and *C* shows that this

effect of the TNX deficiency is mediated predominantly by preventing a high increase in proximal EDL force at higher TS lengths.

Figure 5 Changes in proximo-distal extensor digitorum longus (EDL) force differences with changes in length of antagonistic or synergistic muscles.

A: Effect of changing triceps surae (TS) muscle-tendon complex length. **B:** Effects of m.tibialis anterior-m. extensor hallucis longus complex (TA+EHL) length. Δl(m+t) is the symbol used to indicate change of muscle tendon complex length expressed with respect to optimum length. The EDL proximo-distal total force difference is plotted. Note that any such difference is indicative of *net* myofascial force transmission between EDL and surrounding muscular and / or non-muscular tissues. For WT mice, note that with increasing TS length, as well as TA+EHL length, this EDL proximo-distal force difference decreases rapidly to zero to change sign (i.e. loading direction), between ℓ_o - 3.5 < l < ℓ_o - 4. At higher lengths this distally directed load increases substantially with further increasing lengths for WT mice. In contrast, for *Tnxb* KO mice (TNX-KO), this proximo-distal EDL force difference only deceases gradually (to a value of ΔF = 0 at highest TS lengths, indicating no *net* myofascial force transmission). Note that this means that for *Tnxb* KO mice myofascial loading direction of EDL is not changed at all over the whole length range studied. The inserts illustrate schematically the direction of the net myofascial loads on EDL, where P and D indicate proximal and distal directions, respectively.



Effects of TA+EHL length change on forces exerted by antagonistic muscles at constant length

(a) Effects on TS

ANOVA showed significant main effects (TA+EHL length, TNX deficiency) on active forces exerted by antagonistic TS, while the length of this muscle was kept at unchanged and relatively short, as well as significant interaction between effects of length and TNX deficiency. Normalized active force decreased substantially (by max. 40% of initial force) as a function of increasing TA+EHL length (*Figure 6A*). The effect of TNX deficiency is to limit the length dependent decrease in TS active force to levels not exceeding 7.4 % of initial force.

(b) Effects on EDL

Proximal force

ANOVA showed significant main effects (of TS length and TNX deficiency) on EDL proximal active force, but no interaction. Note that, at low lengths, proximal active force increases (max. increase > 0.1 N, TA+EHL = -1 mm at) as a function of increasing TA+EHL length, but remains at similar values for higher TA+EHL lengths. This effect is still present in *Tnxb* KO mice, but occurs at lower force levels (*Figure 6B*): the curve is shifted downward.

Distal force

ANOVA showed significant effects of TA+EHL length on EDL distal active force, as well as significant interaction of effects of TS length and TNX deficiency. Note that distal active force decreases as a function of increasing TA+EHL length (*Figure 6C*) in both WT mice and *Tnxb* KO mice. However, such decrease in active force is much smaller in *Tnxb* KO mice.

Proximo-distal EDL total force differences

ANOVA showed significant main effects on the EDL total proximo-distal force difference, as well as significant interaction of effects of TS length and TNX deficiency. Therefore, it is concluded that TA+EHL length and TNX deficiency affect myofascial force transmission within the mouse lower leg. Similar as for TS length change, the pattern of effects of TA+EHL length changes show the following: At low lengths, the force difference is always positive for both WT and *Tnxb* KO mice indicating that a net load is exerted on EDL from proximal direction. In both cases this load decreases to very low values with increasing TA+EHL lengths. However for WT mice (*Figure 5B*), this occurs after minor TA+EHL length increase (≈ 0.6 mm) after which the direction of loading is reversed and increases progressively with further length increases. For *Tnxb* KO mice the proximally directed net myofascial load decreases much more gradually to levels approaching zero at high TA+EHL lengths, i.e. the reversal of loading direction is absent.

(c) TS and TA+EHL length-force characteristics

ANOVA showed a significant main effect (i.e. for factor length) for both TS (*Figure 7A*) and TA+EHL (*Figure 7B*) active, as well as passive length –force curves. However, despite non-overlapping curves, any possible effects of TNX deficiency, as well as its interaction with length could not be shown to be significant (ANOVA) for either muscle due to a relatively high individual variation of force values.

Figure 6 Effects of changes in m.tibialis anterior - m.extensor hallucis longus complex length on forces exerted by antagonistic muscles kept at constant length.

A: Force exerted by m. triceps surae (TS) with changing lengths of m. tibialis anterior m. extensor hallucis longus complex (TA+EHL). Normalized active force (Fma) decreased substantially (by max. 40% of initial force) as a function of increasing TA+EHL length. The effect of TNX deficiency is to limit this length dependent decrease in TS active force to levels not exceeding 7.4 % of initial force. **B:** Proximal force exerted by m. extensor digitorum longus (EDL) with changing lengths of TA+EHL. Proximal active force (Fma prox) increases as a function of increasing TA+THL length at low lengths, but remains at similar values for higher TA+EHL lengths. This effect is still present in *Tnxb* KO (TNX-KO) mice, but occurs at lower force levels: the curve is shifted downward. **C:** Distal force exerted by (EDL) with changing lengths of TA+EHL. Distal active force (Fma dist) decreases as a function of increasing TA+EHL length in both wildtype (WT) mice and *Tnxb* KO mice. However, such decrease in active force is much smaller in *Tnxb* KO mice. $\Delta\ell$(m+t) is the symbol used to indicate change of muscle tendon complex length expressed with respect to optimum length.

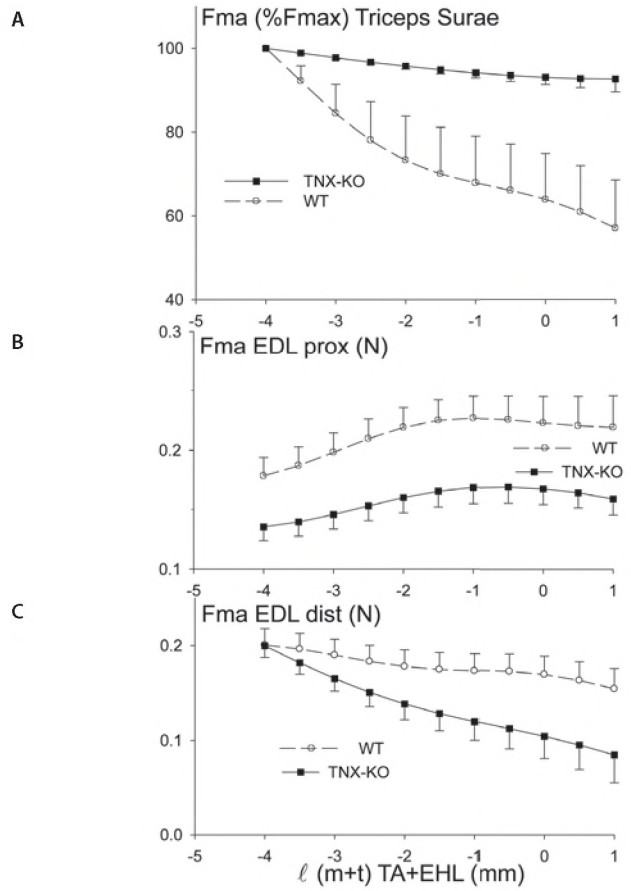
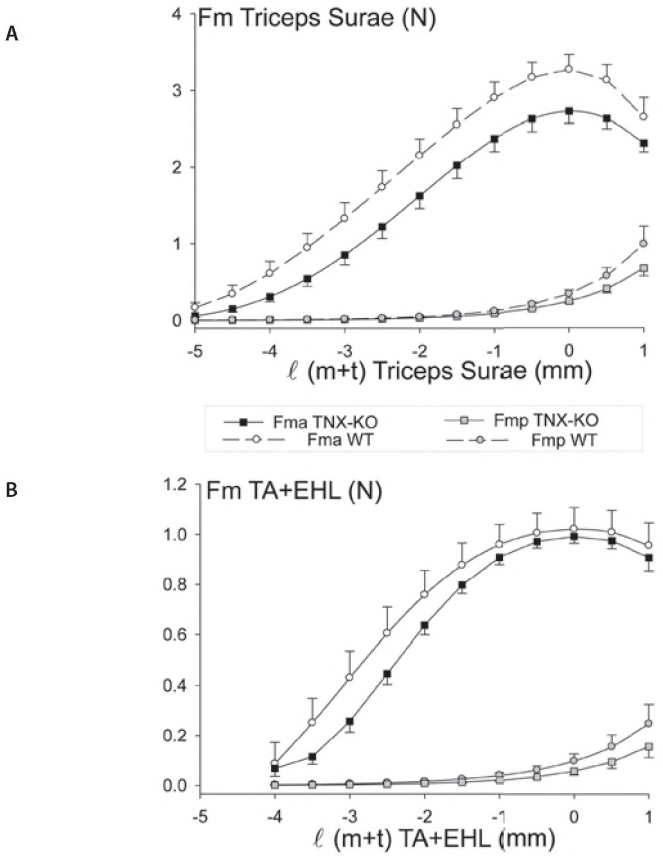


Figure 7 Triceps surae (TS) and m.tibialis anterior--m.extensor hallucis longus complex (TA+EHL) length-force characteristics.

A: TS active and passive length-force curves for *Tnxb* KO mice (TNX-KO) and for the WT mice. **B:** TA+EHL active and passive length-force curves for TNX knockout mice (TNX-KO) and for the WT mice. Note that no significant differences could be proved for these curves between *Tnxb* KO mice and WT mice. $\Delta\ell$ (m+t) is the symbol used to indicate change of muscle tendon complex length expressed with respect to optimum length.



Discussion

The results of this study show that altered properties of any series elastic component of muscle due to TNX deficiency directly affect muscular characteristics. More specifically, study of the intramuscular aspects (*Series A*) points to changes in the series elastic component within the (maximally dissected) muscle-tendon complex and study of muscle within its connective tissue context showing altered mechanical interaction between muscles (*Series B*) points at altered compliance of connective tissues located outside the individual muscles.

Mechanical interaction between antagonistic and synergistic muscles due to myofascial force transmission has been described in the last few years.[5,18-20] Altered intra- and epimuscular myofascial force transmission may drastically affect muscular coordination required for physiological movements. Main findings are summarized below.

Series A. Intramuscular changes: increased muscle compliance

At optimum length (ℓ_O) several properties of dissected GM are unchanged in *Tnxb* KO mice compared to WT mice: 1) maximal isometric force and maximal power production; 2) stimulation frequency-force relationship; and 3) fatigability during a series of repeated isometric contractions. In contrast, at low lengths (ℓ_O– 4, ℓ_O– 3.5, and ℓ_O– 3), some GM properties were affected significantly in *Tnxb* KO mice: 4) active force exerted at lower lengths was lower; 5) the maximal rate of relaxation was lower; 6) the time delay between first stimulation pulse and the time of attainment of 2% of maximal active force was longer in *Tnxb* KO mice. This last finding is not directly related to our hypothesis, but indicates that relatively more slack must be taken up at lower lengths in *Tnxb* KO mice. The other findings are related to the higher series elastic compliance present in the disease, which causes more shortening to be imposed on the muscle fibres in *Tnxb* KO mice at the onset of contraction at low lengths. Vice versa, more lengthening is imposed on the muscle fibre at the onset of relaxation at low lengths. These findings are in accordance with the results of our previous pilot study in two TNX-deficient EDS patients, which were only tested at long length.[5]

Series B. Intermuscular effects: reduced myofascial interaction between synergistics and antagonistic muscles

TNX deficiency strongly affects the mechanical interaction between muscles, which reflects the reduction of epimuscular myofascial force transmission (i.e. transmission directly between muscle belly and its surrounding tissues). The decrease in normalized distal active force in their agonistic muscle (TA+EHL and TS respectively) with increasing length of the antagonistic muscle (TS and TA+EHL respectively) in normal mice results from myofascial force transmission between these muscle groups. The effect of TNX deficiency is to limit this antagonist-length dependent decrease in active force, which is compatible with the

hypothesis of increased compliance of tissues surrounding the individual muscles. Second, the difference in force exerted at proximal and distal tendons of EDL is indicative of net epimuscular myofascial force transmission between EDL and other muscular or non-muscular tissues.

This difference also proved to be a function of the antagonistic or synergistic muscle-tendon complex length (TS and TA+EHL respectively) and was affected by TNX deficiency. The deficiency significantly affects *net* epimuscular myofascial force transmission, not only in the magnitude of the *net* myofascial load on EDL, but also with regard to the direction of loading. Whereas in WT mice the direction changes rapidly from proximal to distal to distal loading of EDL with increasing TS length, such change of direction does not occur in *Tnxb* KO mice. In other words, in comparable experimental conditions *Tnxb* KO muscles act more independently than healthy muscles. It seems inevitable that such altered function will require altered patterns of muscular coordination to allow effective movement.

The structure that is most likely responsible for a proximal load is the neurovascular tract (i.e. the connective tissues reinforcing blood vessels and nerves outside of the muscle).[19] This load and loading direction is thought to be present permanently (unless the muscle is lengthened proximally,[20] note that this is only possible in polyarticular muscles). The similarity of proximal loads on EDL (see *Figure 4* at low lengths for TA+EHL and TS) is hypothesized to indicate that the compliance of the neurovascular tract to this muscle is not affected in a major way by the TNX deficiency. This would agree with the clinical observation that EDS patients with TNX deficiency, in contrast to other types of EDS, do not suffer from major damage to blood vessels and nerves. In healthy animals, as the synergistic or antagonistic muscles are lengthened, a distally directed myofascial load on EDL is enhanced and compensates for proximally directed load at slightly higher lengths (change of net loading direction) and becomes dominant (net distally directed load) at even higher lengths. In contrast, for *Tnxb* KO mice enhanced compliance of collagenous tissues connecting EDL to synergistic or antagonistic muscles necessitates much higher length changes of these muscles to even attain equilibrium between the opposing myofascial loads on EDL at very high lengths, let alone attaining a change in direction of loading.

Conclusion

Taken together, these findings indicate that the series elastic components of the muscle-tendon complex located within and between muscles is changed in *Tnxb* KO mice. As such, these findings support the hypothesis formulated previously that TNX deficiency reduces the stiffness of myofascial pathways and thus causes a pathological reduction of the force transmitted this way.[5] This study and previous animal experiments have shown that myofascial force transmission occurs between antagonistic muscles, which points to the high interdependence of muscles and their role in higher levels of motor organization.[19,20]

Whether and to what extent reduced myofascial force transmission changes the muscular coordination and interferes with mechanical interaction between antagonists muscles in TNX-deficient EDS patients needs to be studied in detail.

In addition, the results of the present study constitute a new type of evidence supporting the concept of myofascial force transmission: altered ECM elastic properties affect quantity and quality of myofascial force transmission. So far, evidence of myofascial force transmission was based upon use of physiological animal models, or on experiments in human patients suffering from spastic paresis.[19]

Altered myofascial force transmission in *Tnxb* KO mice is likely to be related to TNX deficiency. TNX is abundantly expressed in various tissues during embryonic development, among which are tendons and perimysium of skeletal muscle.[21,22] In adulthood, TNX is predominantly expressed in connective tissue of skeletal and cardiac muscle.[22] TNX is involved in collagen deposition and maturation,[2,23] and several studies suggest that TNX acts as a bridge between collagen fibrils and as such may be important for the compliance of connective tissues.[24] Firstly, TNX is located between collagen fibrils organized in bundles[25] and the interfibrillar distance is increased in skin of TNX-deficient patients. Furthermore, TNX was found to assemble into disulfide-linked oligomers, of which trimers are the predominant form. This disulfide-linked trimer structure of TNX is a property that is probably important for bridging.[24] TNX interacts with types I, III and V fibrillar collagen molecules and with decorin, and binds to the fibril-associated types XII and XIV collagens.[24,26] Finally, the FNIII domains of TNX may be important for elastic properties of the molecule.[24]

In short, altered muscular function in *Tnxb* KO mice is partially explained by changes of series elastic components of the muscle-tendon complex, which results in altered intra- and epimuscular myofascial force transmission. We hypothesize that this direct effect of altered ECM composition contributes to muscle weakness in EDS patients, in addition to mild myopathic effects of the disease on muscular histology.

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
3. Makareeva E, Cabral WA, Marini JC, Leikin S. Molecular mechanism of alpha 1(I)-osteogenesis imperfecta/ Ehlers-Danlos syndrome: unfolding of an N-anchor domain at the N-terminal end of the type I collagen triple helix. J Biol Chem 2006; 281: 6463-6470.
4. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
5. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
6. Voermans NC, Timmermans J, van Alfen N, Pillen S, op den Akker J, Lammens M, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular features in Marfan syndrome. Clin Genet 2009; 76: 25-37.
7. Huijing PA. Epimuscular myofascial force transmission: a historical review and implications for new research. International Society of Biomechanics Muybridge Award Lecture, Taipei, 2007. J Biomech 2009; 42: 9-21.
8. Huijing PA, Baan GC, Rebel GT. Non-myotendinous force transmission in rat extensor digitorum longus muscle. J Exp Biol 1998; 201: 683-691.
9. Maas H, Baan GC, Huijing PA. Intermuscular interaction via myofascial force transmission: effects of tibialis anterior and extensor hallucis longus length on force transmission from rat extensor digitorum longus muscle. J Biomech 2001; 34: 927-940.
10. Yucesoy CA, Koopman BH, Baan GC, Grootenboer HJ, Huijing PA. Extramuscular myofascial force transmission: experiments and finite element modeling. Arch Physiol Biochem 2003; 111: 377-388.
11. Huijing PA. Muscular force transmission necessitates a multilevel integrative approach to the analysis of function of skeletal muscle. Exerc Sport Sci Rev 2003; 31: 167-175.
12. Mao JR, Taylor G, Dean WB, Wagner DR, Afzal V, Lotz JC, Rubin EM, Bristow J. Tenascin-X deficiency mimics Ehlers-Danlos syndrome in mice through alteration of collagen deposition. Nat Genet 2002; 30: 421-425.
13. Egging DF, van Vlijmen I, Starcher B, Gijsen Y, Zweers MC, Blankevoort L, Bristow J, Schalkwijk J. Dermal connective tissue development in mice: an essential role for tenascin-X. Cell Tissue Res 2006; 323: 465-474.
14. de Haan A, Jones DA, Sargeant AJ. Changes in velocity of shortening, power output and relaxation rate during fatigue of rat medial gastrocnemius muscle. Pflugers Arch 1989; 413: 422-428.
15. de Haan A, de Ruiter CJ, Lind A, Sargeant AJ. Age-related changes in force and efficiency in rat skeletal muscle. Acta Physiol Scand 1993; 147: 347-355.
16. Huijing PA, Baan GC. Extramuscular myofascial force transmission within the rat anterior tibial compartment: proximo-distal differences in muscle force. Acta Physiol Scand 2001; 173: 297-311.
17. Neter J, Wasserman W, Kutner ME. Applied linear statistic models: regression. analysis of variance and experimental design. Irwin, Homewood; 1990.
18. Carson RG. Changes in muscle coordination with training. J Appl Physiol 2006; 101: 1506-1513.
19. Huijing PA. Epimuscular myofascial force transmission between antagonistic and synergistic muscles can explain movement limitation in spastic paresis. J Electromyogr Kinesiol 2007; 17: 708-24.
20. Huijing PA, Baan GC. Myofascial force transmission via extramuscular pathways occurs between antagonistic muscles. Cells Tissues Organs 2008; 188: 400-414.
21. Burch GH, Bedolli MA, McDonough S, Rosenthal SM, Bristow J. Embryonic expression of tenascin-X suggests a role in limb, muscle, and heart development. Dev Dyn 1995; 203: 491-504.
22. Matsumoto K, Saga Y, Ikemura T, Sakakura T, Chiquet-Ehrismann R. The distribution of tenascin-X is distinct and often reciprocal to that of tenascin-C. J Cell Biol 1994; 125: 483-493.

23. Egging D, van Vlijmen-Willems I, van Tongeren T., Schalkwijk J, Peeters A. Wound healing in tenascin-X deficient mice suggests that tenascin-X is involved in matrix maturation rather than matrix deposition. Connect Tissue Res 2007; 48: 93-98.
24. Lethias C, Carisey A, Comte J, Cluzel C, Exposito JY. A model of tenascin-X integration within the collagenous network. FEBS Lett 2006; 580: 6281-6285.
25. Lethias C, Descollonges Y, Boutillon MM, Garrone R. Flexilin: a new extracellular matrix glycoprotein localized on collagen fibrils. Matrix Biol 1996; 15: 11-19.
26. Elefteriou F, Exposito JY, Garrone R, Lethias C. Binding of tenascin-X to decorin. FEBS Lett 2001; 495: 44-47.

CHAPTER 10

Influence of tenascin-X deficiency on muscular properties of the thigh muscles: a quantitative study in Ehlers-Danlos syndrome patients

Adapted from:
Gerrits KH, Voermans NC, de Haan A, van Engelen BG.
Submitted.

Abstract

The Ehlers-Danlos syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Muscle involvement in EDS can be expected based on interactions between muscle and ECM molecules (such as collagen I, III, V, and tenascin-x (TNX)). A recent study in *Tnxb knockout* mice showed reduced quantitative muscle function due to an increased compliance of the series elastic component. This calls for confirmation in TNX-deficient EDS patients.

We therefore performed quantitative muscle function measurements at different joint angles and evaluated voluntary activation capacity in seven TNX-deficient EDS patients.

The main findings were that: 1) TNX-deficient EDS patients exhibited reduced maximal voluntary torque of the knee-extensors across all joint angles tested (at 30 °, 60 °, and 90 ° knee flexion), while no differences were found on MVTs of knee flexors; 2) the normalized maximal rate of torque development did not differ between the groups, but the time to reach this normalized maximal rate of torque development was delayed in TNX-deficient patients, especially at 30 °; 3) normalized torques (normalized to the highest torque at 60 °) were not different between EDS patients and controls at 30 ° and tended to be higher in EDS patients at 90 °; and 4) EDS patients exhibited reduced voluntary activation capacity, particularly at low muscle length as compared to controls. Importantly, these results could not be explained by a difference in level of physical activity.

In short, isometric voluntary peak torque is reduced in TNX-deficient patients, due to an increased compliance of the series elastic component of muscle tissue and to a failure to maximally voluntary activate the muscles.

Introduction

The Ehlers-Danlos syndrome (EDS) is a clinically and genetically heterogeneous group of inherited connective tissue disorders (ICTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility.[1] Mutations in type I and V collagen can explain part of the classical type EDS cases, and the vascular type is caused by mutations in the gene encoding collagen III. A clinically distinct recessive form results from deficiency of tenascin-X (TNX), another extracellular matrix molecule (ECM) that is expressed in muscle.[2,3]

Muscle involvement in EDS can be expected based on interactions between muscle and ECM molecules known to be deficient in EDS, such as collagen I, III, V, and TNX.[4] In fact, muscle hypotonia and muscle rupture are diagnostic criteria of EDS, and fatigue, musculoskeletal pain, and delayed gross motor development are described as associated features in these criteria.[1] However, muscle symptoms in EDS have long been interpreted to result from exercise avoidance due to joint hypermobility and instability,[5] and reports on neuromuscular function in EDS have been sparse until recently.[6-9]

Inspired by a number of EDS patients at our outpatients department and the interactions mentioned above, we performed a systematic observational study on neuromuscular features in TNX-deficient and other types of EDS (n = 40). The results showed muscle weakness in most patients (85%), with signs of mild myopathy and / or axonal polyneuropathy.[10] Overall, patients with the hypermobility type EDS caused by *TNXB* haploinsufficiency (with reduced TNX serum levels) were less affected than the TNX-deficient EDS patients (with complete absence of TNX). This pointed to a relation between residual TNX level and degree of neuromuscular involvement, compatible with a dose-effect relation. The interaction between ECM molecules involved in EDS (collagen I, III, V, and TNX) and the transsarcolemmal molecules,[4] the finding of reduced collagen fibril density in muscle of TNX-deficient EDS patients,[10] and the dose-effect relation described above[10] suggest that the ECM defect in EDS influences muscle function.

The results of our pilot study on quantitative muscle function in EDS patients were indeed compatible with the hypothesis of a role of the ECM in muscle function.[11] This study in two TNX-deficient EDS patients measured knee extension torques at relatively long muscle length (90° knee flexion; longer than optimum muscle length). It showed reduced maximal torques at voluntary contraction, normal torque variation with 10 Hz stimulation, relatively high twitch torques compared to tetanic torques at 150 Hz stimulation, and a normal delay between electrical stimulation and torque generation. Physical examination, muscle ultrasound, and muscle biopsy had revealed no signs of atrophy. Hence, the findings could not be attributed to reduced physical activity or muscle atrophy. Further, it seemed unlikely that the reduced muscle function resulted from increased tendon compliance. This would have reduced torque generation, have lowered the twitch torques, have increased the delay

between stimulation and torque generation, and have delayed relaxation. On the other hand, muscle function was studied at a relatively long muscle length (i.e. 90 ° knee-flexion), which may have masked the effects of increased tendon compliance. We therefore hypothesized that quantitative muscle function in EDS was lowered due to a reduction of myofascial force transmission secondary to the ECM defect.[11]

Our subsequent quantitative muscle function study in *Tnxb* knockout (KO) mice indeed showed differences only at low muscle lengths (optimum length - 4, - 3.5, - 3 mm). The normalized active isometric force was lower; the delay between stimulus and attainment of 2% of maximal active force (i.e. the electromechanical delay) was longer; and the relaxation rate was reduced. These parameters were all normal at optimum length and above. This pointed to changes in the series elastic component within the maximally dissected GM muscle-tendon complex, which implies the ECM network of both endo- and perimysium within the muscle and the myotendinous pathway.[12]

These experiments at short muscle length added two important findings to the results of the pilot study in EDS patients described above. First, the altered muscle properties at short muscle length pointed to an increased compliance of the series elastic component, whereas in the pilot study with measurements at relatively long muscle length (90° knee flexion) no such observations were made. Changes of series elastic components would indeed be expected to manifest first at short muscle length, since more slack has to be taken up at the onset of contraction before the series elastic component can transmit forces. Second, normalized active isometric force after electrical stimulation in *Tnxb* KO mice was normal at optimum length and above, whereas maximal torque at voluntary contraction in TNX-deficient EDS patients was reduced at relatively long muscle length. This difference raised the question whether reduced voluntary activation capacity contributes to muscle weakness in EDS patients, as we previously reported in neuromuscular diseases (fascioscapulohumoral dystrophy, mytonic dystrophy, hereditary motor sensory neuropathy).[13]

These findings call for confirmation in TNX-deficient EDS patients, with an experimental protocol including quantitative muscle function measurements at different joint angles and evaluation of voluntary activation capacity. Therefore, the purpose of the present study was to test the hypotheses that 1) isometric peak torque (of maximal voluntary contraction); that 2) rate of torque development (of triplet torque) of knee extensor muscles of TNX-deficient patients is lower compared to control subjects; and that 3) this is more pronounced at low knee flexion (i.e. relatively short muscle length) compared to high knee flexion (i.e. relatively long muscle length). Furthermore we tested 4) whether voluntary activation capacity is reduced in TNX-deficient patients and whether this also depends on knee joint angle. Finally, we evaluated current physical activity of patients and control subjects to exclude that the findings of this study could be influenced by differences in physical activity.

Methods

Subjects

We invited all ten TNX-deficient EDS who had previously participated in our clinical study on neuromuscular features in EDS.[10] All were willing to participate; but three patients were excluded: one due to pregnancy; one due to admittance to the ICU at the time of the study; and one since she could not tolerate the electrical stimulation during the study. The age- and sex matched controls consisted of three friends of the patients; and the other two were recruited among the university staff.

Median age of the seven patients included was 42 years (SD 16), and four of them were female; median age of the controls was 43 years (SD 16). TNX-deficient EDS was diagnosed by a medical specialist in all patients, based on clinical features described by Schalkwijk et al. and confirmed by complete absence of TNX in serum.[3] *TNXB* mutation analysis was performed in four patients; revealing a heterozygous 30 kB deletion in two patients, in whom the second TNX mutation had not found, and a homozygous 2 bp deletion (exon 8) in two other patients. These four patients were previously reported by Schalkwijk et al.(Patient 1 and her sister; patient 2 and her brother).[3] The study was approved by the medical ethical committee of the Radboud University Nijmegen medical centre.

Experimental design

All measurements were performed on one day at the Research Institute MOVE.

All subjects were asked to refrain from strenuous exercise for 48 h prior to the experiments. To be able to control for level of physical activity, we used the International Physical Activity Questionnaire (IPAQ; see below). To assess maximal voluntary torque (MVT), voluntary activation capacity, and rate of torque rise of the right thigh muscles, subjects performed isometric voluntary (both knee-extensors and -flexors) as well as electrically elicited (knee-extensors only) contractions at 30 °, 60 ° and 90 ° knee flexion (0 ° corresponds with full extension). Before experiments started subjects were familiarized with the test procedures such as electrical stimulation. Furthermore, subjects were trained to perform a maximal voluntary contraction of approximately 3 - 5 s duration. Knee extension and flexion torques were measured with the subjects seated on a custom-built computer-controlled lower limb dynamometer. The lower leg was connected to the lever arm of the dynamometer with the hip flexed at approximately 60 ° and the knee flexed at different angles. Padded straps around the pelvis and upper body minimized undesired movements of the hip during the measurements. Furthermore, care was taken that the axis of the lever arm was always aligned with the axis of the knee joint (lateral femur condyl). Knee extension and flexion torques (0.001 Nm resolution) were measured at the motor axis and are therefore independent of the length of the lever arm. Torque signals were digitized (1000 Hz) and stored on disc for immediate and off-line analysis.

Assessment of physical activity

To evaluate current physical activity, all subjects were asked to fill in the long form of the IPAQ.[14] This written questionnaire assesses physical activity undertaken across a comprehensive set of domains including: leisure time physical activity, domestic and gardening (yard) activities, work-related physical activity, and transport-related physical activity. The items in the questionnaire are structured to provide separate scores on these domains. The total score is expressed as MET minutes per weeks (METs are multiples of the resting metabolic state, and a MET minute is computed by multiplying the MET score of an activity by the minutes it is performed). This requires recording of duration (in minutes) and frequency (days per week) of walking, moderate-intensity- and vigorous-intensity activities. The following values were used for analysis of the IPAQ data: walking 3.3 METs; moderate physical activity 4.0 METs, and vigorous physical activity 8.0 METs. Using these values, scores were calculated for each domain. The sum of activities in the four domains represents the total weekly physical activity for each subject (MET – minutes/week scores). Based on these calculations, three categorical scores were arbitrarily defined: low (< 600 METs per week), moderate (600 - 3000 METs per week), and high (≥ 3000 METs per week).(Online available guidelines of the IPAQ: http://www.ipaq.ki.se/scoring.pdf).

Torque measurements

Numbers in brackets correspond with the aims in the introduction.

Maximal voluntary torque at various muscle lengths (1;3)

Similar procedures were followed at each of the three knee angles tested (30 °, 60 °, and 90 ° of knee flexion). We always started at 60 ° because pilot experiments have shown that the electrical current needed to evoke maximal contractions (see below) was highest at this position. The other two angles were randomly assigned. First, subjects were asked to perform two to four maximal voluntary knee extensions and flexions, each separated by 2 min rest. The highest torque produced during these attempts was defined as MVT. During each voluntary effort subjects received substantial verbal encouragement and visual feed-back to achieve maximal performance. To minimize the total number of maximal contractions, possibly leading to muscle fatigue, an extra attempt was made only if the last attempt was > 10% higher than the previous. MVTs were normalized to the MVT at 60 ° knee flexion (nMVT).

Maximal triplet torque and voluntary activation capacity (4)

A modified super-imposed stimulation technique was used to estimate the voluntary activation capacity (i.e. the degree of maximal voluntary activation) during each attempt. Self-adhesive surface electrodes (8 x 13cm, Schwa-Medico B.V., Nieuw Leusden, the Netherlands) were placed over the medial distal part and the lateral proximal part of the quadriceps muscle. A constant current electrical stimulator (model DS7A, Digitimer Ltd,

Hertfordshire, UK) imposed a brief train of 3 electrical pulses (triplet) at 300 Hz (pulse width: 0.2 ms) on the relaxed quadriceps muscle. Subsequently, the same triplet was imposed on top of the plateau phase of the maximal voluntary contraction. The stimulation current for the triplets was set at supra-maximal intensity to improve the signal-noise ratio. Most subjects could tolerate these stimulations, most likely due to the short duration of the stimulation. The maximal triplet torque was used as an estimate of torque capacity independent of the degree of voluntary activation. The voluntary activation capacity during a maximal voluntary contraction was estimated calculating the 'voluntary activation index' with the equation:

$$\text{Voluntary activation index (\%)} = \left[1 - \frac{\text{Superimposed triplet torque}}{\text{Control triplet torque}} \right] * 100\%$$

where the superimposed triplet torque is the extra torque produced by the triplet on top of the maximal voluntary contraction and the control triplet torque is the torque produced by the same triplet imposed on the relaxed muscle. To estimate the maximal torque capacity (MTC; voluntary torque which would have been possible when voluntary activation was maximal) the MVT was corrected with the voluntary activation index of the knee-extensor muscles.

Rate of torque development (2)

From the torque differential of the supramaximal triplet stimulation we calculated the maximal rate of torque development (MRTD) which was then normalized to the peak torque of that particular triplet (normalized MRTD = nMRTD). The time to MRTD (tMRTD) was defined as the time from start of stimulation to the moment at which MRTD was reached.

Data analysis and statistics

Off-line analysis of torque recordings was performed with applications using custom Matlab software packages. To examine differences in the MVT, triplet torque, voluntary activation index, MTC, MRTD and tMRTD with varying joint angle between patients and control subjects, repeated- measures factorial analyses of variance (ANOVAs) were performed and the interaction between group and angle was examined. Post hoc simple (subject group) and repeated (angle) contrast analysis was used to study differences between repeated measures. All data are presented as mean ± SD unless otherwise indicated, and levels of significance were set at $P < 0.05$.

Table 1 Biomechanical concepts in this study.

Term definition	Abbreviation (unit)	Description
Metabolic equivalent	MET	METs are multiples of the resting metabolic state, and a MET minute is computed by multiplying the MET score of an activity by the minutes it is performed.
Maximal voluntary torque	MVT (N.m)	Torque measured at maximal voluntary contraction.
Normalized MVT	nMVT	MVT normalized to the highest torque at 60 ° knee flexion.
Voluntary activation capacity en voluntary activation index	VA (%)	The degree of maximal activation during voluntary contraction; this is measured with the voluntary activation index (with the maximal torque capacity and the superimposed triplet torque as its variable).
Maximal torque capacity	MTC (N.m)	The torque reached after maximal triplet stimulation; this is considered to be independent of the degree of voluntary activation.
Maximal rate of torque development	MRTD (N.m/ms)	maximal rate with which the torque increases after electrical stimulation (supramaximal triplet stimulation).
Normalized MRTD	nMRTD	MRTD normalized to the peak torque of that particular triplet.
Time to reach MRTD	tMRTD (ms)	The time from start of stimulation to the moment at which MRTD is reached.

Results

Assessment of pysical activity

All healthy subjects and all but one patient were classified as " highly active" with a sum score of > 3000 METmin per week. One patient had a sum score of 1559 and was classified as moderately active. The mean sum score was 7988 for the healthy subjects and 8789 for the TNX-deficient EDS patients. *Table 2* shows the weekly MET minutes in the various domains. TNX-deficient EDS patients tended to be physically more active at work, whereas healthy controls were more active in leisure time activities. Differences between the two groups were not statistically significant.

Torque measurements

Maximal voluntary torque at various muscle lengths (1;3)

All subjects were able to perform maximal voluntary contractions and achieved the highest torque output within a few attempts, with no further increase on subsequent trials.

For the knee-extensors across all angles measured, patients showed significantly lower absolute MVTs compared to controls (P = 0.018): 69 ± 19 Nm versus 128 ± 3 5 Nm at 30 °, 115

Table 2 The weekly MET minutes in the various domains of TNX-deficient EDS patients and healthy controls. Weekly MET minutes (minutes per week * intensity).

	Healthy subjects				TNX-deficient EDS patients			
	Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
Work	2777	0	10008	4193	4784	0	10080	4373
Transportation	1408	900	2754	775	1167	0	2772	1026
Gardening and domestic chores	1428	90	5160	2114	1781	0	3645	1383
Leisure time	2375	636	4068	1595	1055	0	2076	713
Total physical activity	7988	5571	13212	3021	8789	1559	15405	5020

± 34Nm versus 207 ± 55 Nm at 60 °, and 106 ± 43 Nm versus 155 ± 66 Nm at 90 °, respectively For the knee flexors no such differences were observed (P = 0.113) (*Figure 1A and B*).

In addition, across groups the torque produced was dependent on knee-flexion angle, P = 0.000 (*Figure 1C and D*). However, the relationship between torque and joint angle of the knee-extensors but in patients tended to be slightly different than that observed in control (P = 0.069). In patients, the reduction of knee-extensor torque from 60 ° to 90 ° tended to be less compared to that observed in controls (P = 0.077). The relationship between torque and joint angle of the knee flexors was not different. (P = 0.525).

Maximal triplet torque and degree of voluntary activation capacity (4)

In contrast with the results for MVT, absolute triplet torque of the knee-extensors was not significantly lower in EDS patients than in controls (P = 0.487): 47 ± 20 Nm versus 51 ± 18 Nm at 30 °, 62 ± 20 Nm versus 70 ± 21 Nm at 60 °, and 51 ± 20 Nm versus 63 ± 24 Nm at 90 °, respectively. Furthermore, the triplet torque was dependent on knee- flexion angle across groups (*Figure 2*) and this torque-angle relation was not significantly different between patients and controls (P = 0.453).

There were substantial and significant differences between groups with respect to the degree of maximal voluntary activation (i.e. voluntary activation capacity measured with the voluntary activation index) of the knee-extensor muscles (*Figure 3*). The control subjects showed high voluntary activation indices across all angles tested, with voluntary activation indices > 90%. Patients were, however, much less capable of reaching these high values, and

scored significantly less compared to controls (P = 0.001). In addition, across groups the voluntary activation index was dependent on knee-flexion angle (P = 0.000) showing a reduced voluntary activation index with lower knee-flexion (i.e. towards knee-extension). This angle dependency differed between groups (P = 0.026), such that this reduction in voluntary activation index at lower knee was more pronounced in the patients.

Rate of torque development (2)

Results of normalized maximal rate of torque development (nMRTD) as well as the time to reach nMRTD (tMRTD) are presented in *Figure 4*. There were no significant differences between the groups (P=0.842) regarding the nMRTD (*Figure 4A*), nor was there any effect of angle (P = 0.258). In contrast, tMRTD was significantly delayed in the EDS patients (P = 0.005, *Figure 4B*). In addition, across groups there was a significant effect of knee-flexion angle (P = 0.007), which was different between patients and controls (P = 0.042). In EDS patients, tMRTD gradually increased with lower knee-flexion, with the longest values at 30 ° (P = 0.010). In contrast, differences between angles seemed less in controls who showed the longest tMRTD at 60 ° (P = 0.016).

Figure 1 (Normalized) maximal voluntary torques (MVT) produced at different knee-flexion angles of knee-extensor (**A** and **C**) and knee-flexor (**B** and **D**) muscle in patients with EDS (black bars) and healthy control subjects (grey bars).

A: Knee extensors: maximal voluntary torques. **B:** Knee flexors: maximal voluntary torques. Error bars reflect SD.

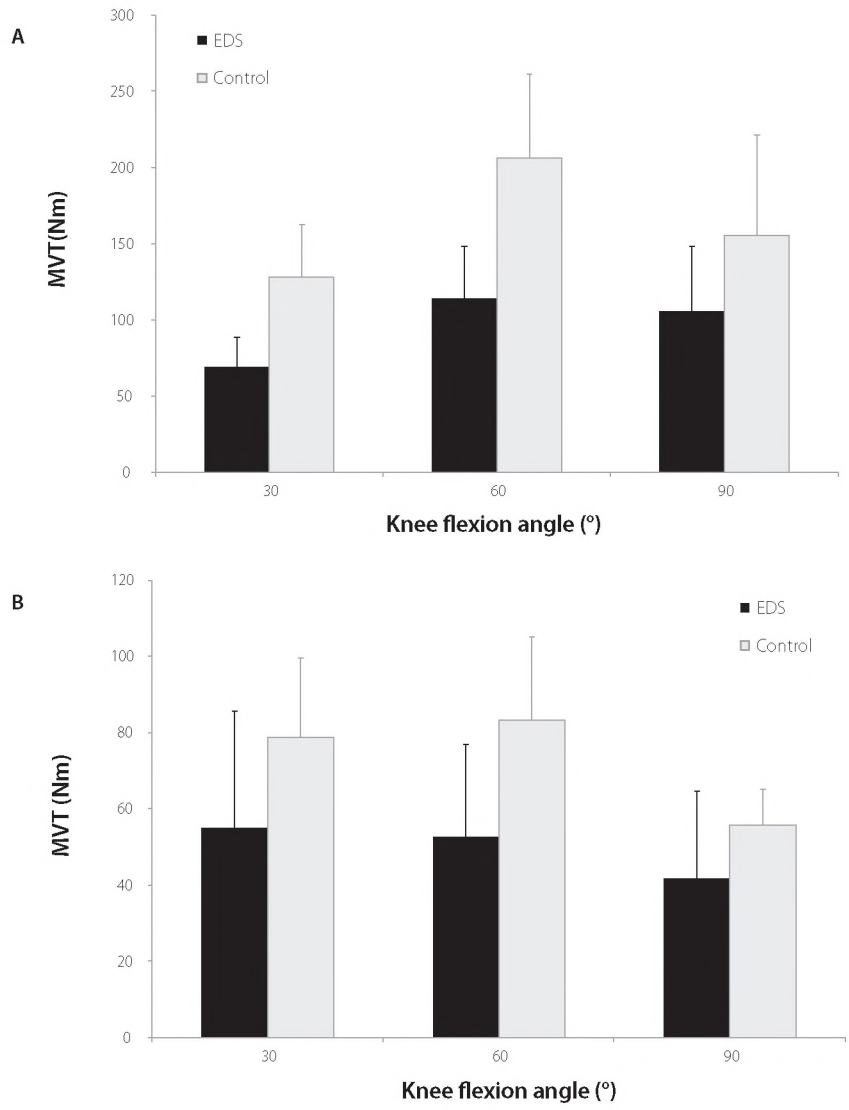


Figure 1 Continued.

C: Knee extensors: normalized maximal voluntary torques. **D**: Knee flexors: maximal voluntary torques. Error bars reflect SD.

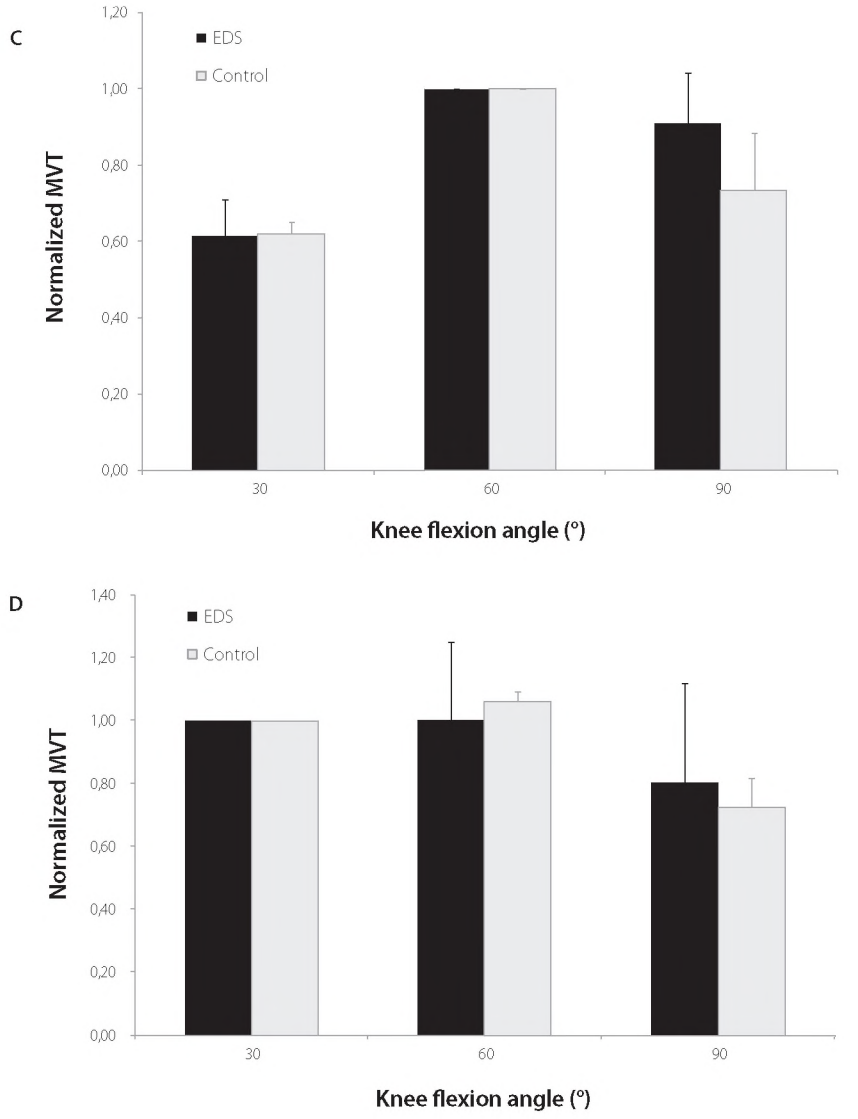


Figure 2 Normalized triplet torque of the knee-extensors.

The absolute triplet torque (TRIP torque) of the knee-extensors was not significantly lower in EDS patients than in controls (*data not shown*). Furthermore, the triplet torque was dependent on knee-flexion angle across groups (normalized TRIP torque; *Figure 2*) and this torque-angle relation was not significantly different between patients and controls (P = 0.453). Error bars reflect SD.

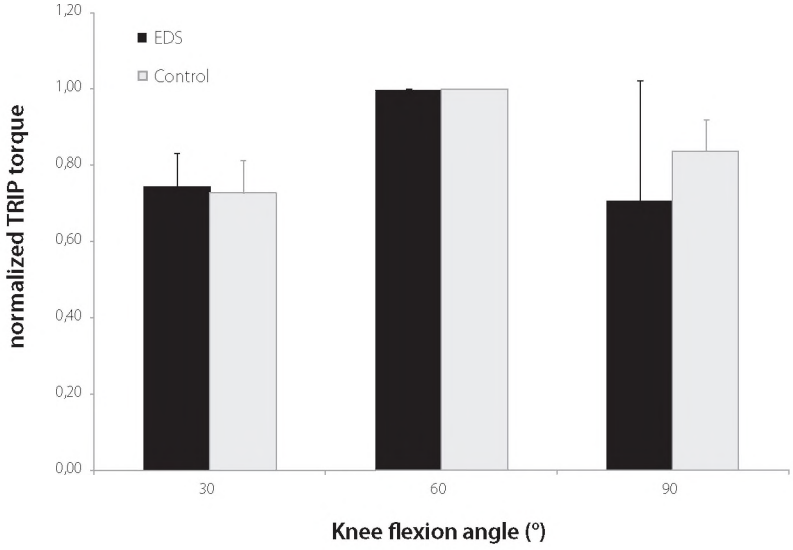


Figure 3 Voluntary activation capacity of knee-extensor muscles.

There were substantial and significant differences between groups with respect to the voluntary activation capacity (measured as the voluntary activation index (VA%)) of the knee-extensor muscles: the control subjects showed high voluntary activation indices across all angles tested, with voluntary activation indices > 90%. Patients were, however, much less capable of reaching these high values, and scored significantly less compared to controls (P = 0.001). In addition, across groups the voluntary activation index was dependent on knee-flexion angle (P = 0.000) showing a reduced voluntary activation index with lower knee-flexion (i.e. towards knee-extension). Error bars reflect SD.

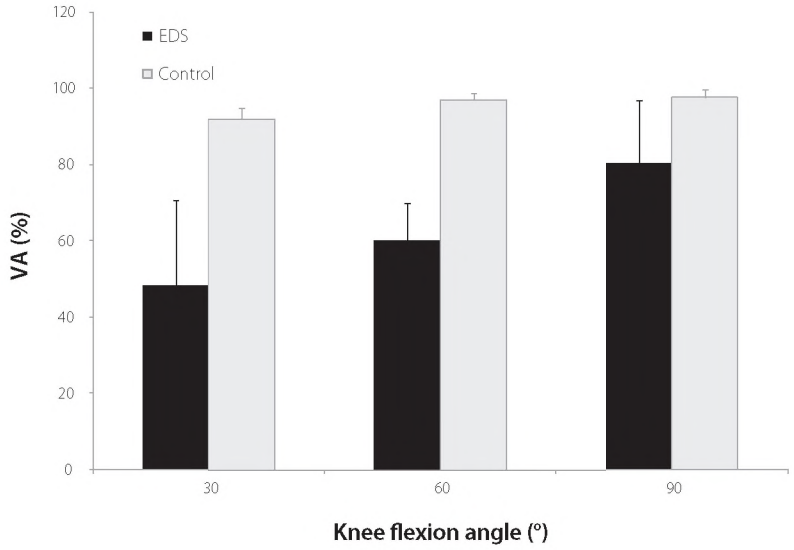
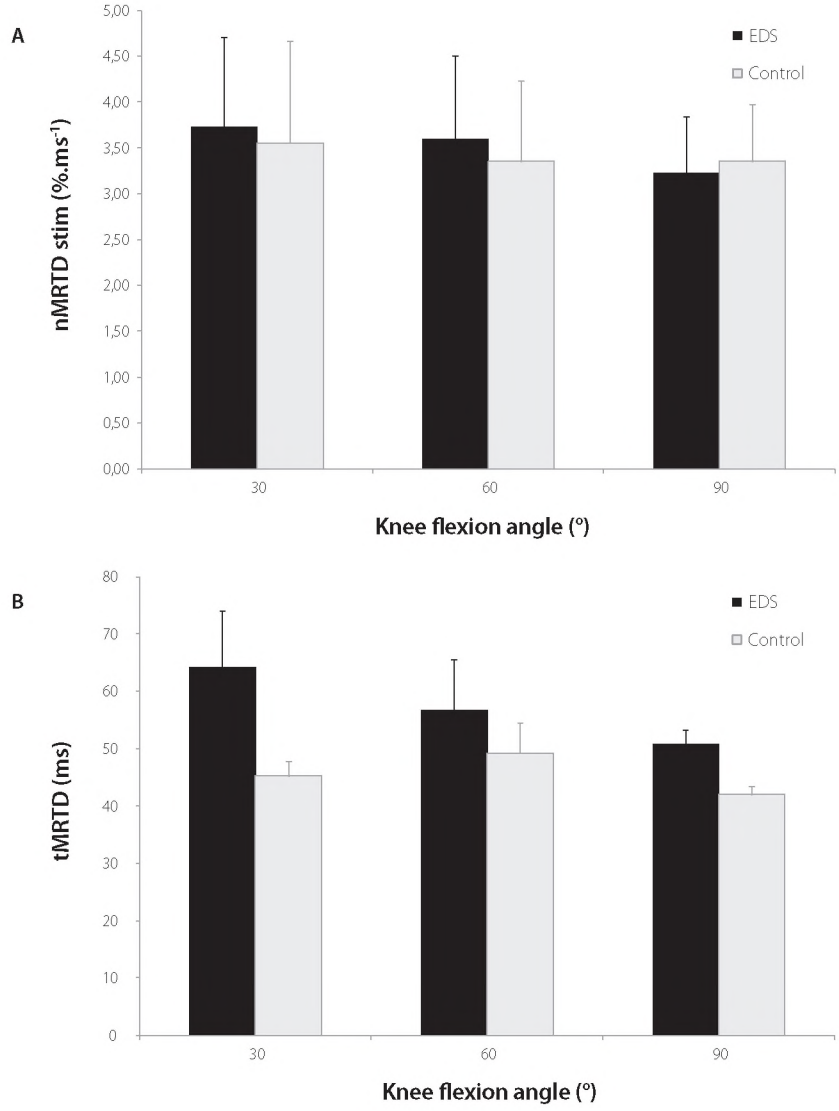


Figure 4 Results of the normalized maximal rate of torque development (nMRTD) as well as the time to reach nMRTD (tMRTD).

A: There were no significant differences between the groups (P = 0.842) regarding the nMRTD, nor was there any effect of angle (P = 0.258) **B:** The tMRTD was significantly delayed in the EDS patients (P = 0.005). In addition, across groups there was a significant effect of knee-flexion angle (P = 0.007), which was different between patients and controls (P = 0.042). In EDS patients, tMRTD gradually increased with lower knee-flexion, with the longest values at 30 ° (P = 0.010). In contrast, differences between angles seemed less in controls who showed the longest tMRTD at 60 ° (P = 0.016). Error bars reflect SD.



Discussion

Background

Our current study was inspired by the combined findings of a pilot study in TNX-deficient EDS patients[11] and the experiments in *Tnxb* KO mice.[12] In the animal model, altered muscle contractile properties were observed only at short muscle length with lower normalized active isometric force, a longer electromechanical delay, and a reduced relaxation rate. These results indicated an increased compliance of the series elastic component. However, the results of the pilot study on quantitative muscle function in TNX-deficient EDS patients had not confirmed this observation. This might be due to the fact that the measurements in TNX-deficient EDS patients were performed at relatively long muscle length, at which possible adaptations in the series elastic component may not have been detectable. Further, the observation in the pilot study that patients showed reduced voluntary torque also at relatively long muscle length may point to a reduction of the maximal voluntary activation capacity in EDS patients.

This present study was therefore designed to assess the influence of TNX deficiency on muscle function in more detail in EDS patients. Further, we aimed to investigate the degree of maximal voluntary activation. We therefore investigated muscle function of knee-extensors and –flexors in EDS patients at different muscle lengths.

The main findings of the study were that: 1) TNX-deficient EDS patients exhibited reduced MVT of the knee-extensors across all joint angles tested (at 30 °, 60 °, and 90 ° knee flexion), while no differences were found on MVTs of knee flexors; 2) the normalized maximal rate of torque development (nMRTD) did not differ between the groups, but the time to reach nMRTD was delayed in TNX-deficient patients, especially at 30°; 3) normalized torques (normalized to the highest torque at 60 °) were not different between EDS patients and controls at 30 ° and tended to be higher in EDS patients at 90 °; and 4) EDS patients exhibited reduced voluntary activation capacity, particularly at low muscle length as compared to controls. Importantly, these results could not be explained by a difference in level of physical activity. We will discuss these findings below (the numbers in brackets correspond with the aims in the introduction).

Maximal voluntary torque production (1)

The muscle weakness observed in the present study (substantial MVT reduction primarily of the knee-extensors) confirms the results of our previous pilot experiment[11] and of our clinical study.[10] First, the two TNX-deficient patients in the pilot study showed significant torque reduction of the knee-extensor muscles at 90 ° knee flexion. Second, the clinical study showed muscle weakness measured by dynamometry in the majority of EDS patients (in 88% of patients the muscle force of the knee extensors measured with dynamometry was below or at the p5 of the normal values).[10,15]

Muscle weakness in EDS has long been suggested to result from reduced physical activities due to exercise avoidance.[1,5] To control for differences in physical activity, we assessed the level of physical activity by a standardized questionnaire (IPAQ). We observed even somewhat (but not statistically significantly) higher average levels of physical activities compared to the control subjects. Based on the results of the present study it seems, therefore, unlikely that reduced physical activity would be responsible for the deteriorated muscle function of the EDS patients. Furthermore, only one of the seven EDS patients included in the present study showed signs of muscle atrophy (assessed with ultrasound measurements during previous experiments).[10] Therefore it is unlikely that muscle atrophy fully accounts for the muscle weakness observed. Instead, this would suggest that TNX deficiency directly influences muscle function.

Influence of knee-flexion angle on maximal voluntary torque production and rate of torque development (2,3)

To obtain a more definite understanding of (the degree of) muscle weakness, we assessed muscle function across a range of joint angles at which the muscle is active during physiological movements. At short muscle length, more slack has to be taken up at the onset of contraction before the series elastic component can transmit forces. Therefore, effects of changes in the series elastic component on torque production will most likely predominate at short muscle length. Hence, assessment of muscle function at relatively short muscle length, in addition to optimum and relatively long muscle length can help to determine whether and to what extent possible changes in the series-elastic component affect muscle function.

Normalized active isometric force in *Tnxb* KO mice was indeed reduced only at short muscle length.[12] This points to increased compliance of the series elastic component of the muscle-tendon complex. Interestingly, and perhaps surprisingly, no reduction in the normalized torque of EDS patients at 30 ° knee flexion was observed. Furthermore at relatively longer muscle length (i.e. 90 ° knee-flexion) normalized torques even tended to be higher in the patients compared with the controls. So, the results of the torque angle-relation do not support the hypothesis of increased compliance of the series-elastic component. In contrast, the observed increase in tMRTD in the TNX-deficient EDS patients does clearly support this hypothesis.

These seemingly conflicting results might have several explanations. First, it is well known that the applied length change of the thigh muscles is relatively low by changing the joint angle of the knee[16] Nevertheless, torque-angle relations have been studied extensively in human knee-extensors and are frequently used as an indirect measure of length-tension relations of these muscles.[17,18] However, the extent of these length changes *in vivo* are likely to be considerably less compared to those applied when studying the length- tension

relationship of a maximally dissected muscle *in situ*. Moreover, if the compliance of the series elastic component of the muscle-tendon complex is indeed increased, the muscle length changes in the EDS patients may even be even less. This is due to the fact that most of the changes in the muscle-tendon complex would be accounted for by length changes of the myotendinous pathway. It may therefore be possible that the range of muscle-length studied in the present study (30 – 60 – 90 ° knee flexion) is simply too narrow to provide evidence for an altered length-tension relationship.

Further, the torque-angle relation in EDS patients differed from that in control subjects with an *increased* normalized torque at 90 ° knee-flexion. This relatively higher torque at higher knee-flexion (i.e. longer muscle length) might be indicative for a shift of the torque-angle relation towards longer muscle length. In this case, the MVT at 60 ° knee-flexion would be below the optimum torque (i.e. the maximum of the torque–angle curve). Consequently, the torque at 30 ° knee-flexion would have been related to a lower than optimum torque. This would lead to an overestimation of the normalized torque at 30 °, thereby explaining the absence of reduced normalized torque at low muscle length. However, we only studied muscle function at three different joint angles, which seems insufficient to provide conclusive evidence for the hypothesized shift in the relationship.

Triplet torques and voluntary activation capacity (2,4)

In contrast with the results for reduced MVT we found no differences in the maximal triplet torques. This suggests that the intrinsic torque generation, independent of voluntary control, is not affected by TNX-deficient EDS patients. In addition, the normalized triplet-angle relations were not different between patients and controls. This finding does not support the above suggested shift in torque- angle relation. However, it should be noted that an increase in the series elastic component could mask possible changes in the torque angle relation. This is especially the case when torques are produced at lower than maximal possible levels. Although triplets were imposed at maximal current intensity, the absolute torque level is still only about half of the maximal possible torques at maximal tetanic torque production.

In the pilot experiment in two TNX-deficient EDS patients,[11] apart from the reduced MVT at 90 °, no signs of an increased series elastic compliance were observed; torque variation with 10 Hz stimulation was relatively normal, we observed relatively high twitch torques compared to tetanic torques at 150 Hz stimulation, and found a normal delay between electrical stimulation and torque generation. It seems that the use of high knee-flexion angle (and thus possibly relatively long muscle length) in that particular study has masked possible effects of increased series elastic compliance.

Finally, the present study clearly showed an impaired capacity of maximal voluntary activation in the EDS patients. This seems somewhat surprising since EDS is not considered to affect central nervous system functioning. A number of studies have shown reduced

voluntary activation capacity in various central nervous system disorders,[19-21] various neuromuscular disorders (fascioscapulohumoral dystrophy, mytonic dystrophy, hereditary motor sensory neuropathy), and chronic fatigue syndrome.[13,22] In fact, fascioscapulohumoral dystrophy is not considered to affect the central nervous system either. The authors suggested that loss of central activation capacity might not only be interpreted negatively. It may also be seen as a positive adaptation which protects the affected muscle against further damage.[23] Furthermore, reduction of physical activity, which is known to affect the voluntary activation capacity[24] cannot explain our observations.

The reduced voluntary activation capacity seems to explain an important part of the torque loss observed during maximal voluntary contractions. However, the extreme loss of voluntary activation capacity with lower knee-flexion, may be partly due to underestimation of voluntary activation capacity. An increase in the compliance of the series elastic component would affect the height of the triplet torque. That is, when compliance is increased, more slack has to be taken up before the series elastic component can transmit forces. This would result in lower 'resting' maximal triplet torques, but not 'superimposed' triplet torques as the latter is imposed on top of an already activated muscle. Since the voluntary activation capacity is calculated by expressing the superimposed triplet relative to the resting triplet, this would lead to an underestimated voluntary activation capacity. Having that in mind, still voluntary activation is also reduced at relatively long muscle length, when the influence of the series elastic component should be negligible. We presently have no explanation for this observation and this may require further investigation.

Final conclusion

The results of the present study show that isometric voluntary peak torque is reduced in TNX-deficient patients. An important part of the observed muscle weakness seems to be explained by failure to maximally voluntary activate the muscles. In addition, the series elastic component of muscle tissue is increased based upon the increased electromechanical delay between stimulus and torque development and the noticeably reduced voluntary activation capacity at low knee-flexion. However, such possible increased compliance seemed not to result in obvious torque reduction at low knee-flexion angle, although measurements of full torque-angel relations may be required to provide conclusive evidence. These findings confirm our observation of mild to moderate neuromuscular involvement in various types of EDS syndrome.[10] The finding of reduced voluntary activation capacity points to a central activation failure, which has been described in various neuromuscular disorders and central fatigue syndrome.[13,22] This calls for further research on central and peripheral fatigue in EDS, results of which explain the high prevalence of severe fatigue among EDS patients.[25]

Reference List

1. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
2. Burch GH, Gong Y, Liu W, Dettman RW, Curry CJ, Smith L, Miller WL, Bristow J. Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. Nat Genet 1997; 17: 104-108.
3. Schalkwijk J, Zweers MC, Steijlen PM, Dean WB, Taylor G, van Vlijmen I, van Haren B, Miller WL, Bristow J. A recessive form of the Ehlers-Danlos syndrome caused by tenascin-X deficiency. N Engl J Med 2001; 345: 1167-1175.
4. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
5. Beighton P. The Ehlers-Danlos syndromes. London: William Heineman Medical Books Limited; 1970.
6. Pretorius ME, Butler IJ. Neurologic manifestations of Ehlers-Danlos syndrome. Neurology 1983; 33: 1087-1089.
7. Banerjee G, Agarwal RK, Shembesh NM, el Mauhoub M. Ehlers Danlos syndrome--masquerading as primary muscle disease. Postgrad Med J 1988; 64: 126-127.
8. Bilkey WJ, Baxter TL, Kottke FJ, Mundale MO. Muscle formation in Ehlers-Danlos syndrome. Arch Phys Med Rehabil 1981; 62: 444-448.
9. Yis U, Dirik E, Chambaz C, Steinmann B, Giunta C. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord 2008; 18: 210-214.
10. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
11. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
12. Huijing PA, Voermans NC, Baan GC, Buse TE, van Engelen BG, de Haan A. Muscle characteristics and altered myofascial force transmission in tenascin-X-deficient mice, a mouse model of Ehlers-Danlos syndrome. J Appl Physiol 2010; 109: 986-995.
13. Schillings ML, Kalkman JS, Janssen HM, van Engelen BG, Bleijenberg G, Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. Clin Neurophysiol 2007; 118: 292-300.
14. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003; 35: 1381-1395.
15. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. J Neurol Neurosurg Psychiatry 1991; 54: 244-247.
16. Herzog W, ter Keurs HE. Force-length relation of in-vivo human rectus femoris muscles. Pflugers Arch 1988; 411: 642-647.
17. Gerrits K, Gommans I, van Engelen B, de Haan A. Quadriceps weakness in a family with nemaline myopathy: influence of knee angle. Clin Sci (Lond) 2003; 105: 585-589.
18. Herzog W, Hasler E, Abrahamse SK. A comparison of knee extensor strength curves obtained theoretically and experimentally. Med Sci Sports Exerc 1991; 23: 108-114.
19. de Haan A, de Ruiter CJ, van der Woude LH, Jongen PJ. Contractile properties and fatigue of quadriceps muscles in multiple sclerosis. Muscle Nerve 2000; 23: 1534-1541.
20. Horstman AM, Beltman MJ, Gerrits KH, Koppe P, Janssen TW, Elich P, de HA. Intrinsic muscle strength and voluntary activation of both lower limbs and functional performance after stroke. Clin Physiol Funct Imaging 2008; 28: 251-261.
21. Gerrits KH, Beltman MJ, Koppe PA, Konijnenbelt H, Elich PD, de HA, Janssen TW. Isometric muscle function of knee extensors and the relation with functional performance in patients with stroke. Arch Phys Med Rehabil 2009; 90: 480-487.
22. Schillings ML, Kalkman JS, van der Werf SP, van Engelen BG, Bleijenberg G, Zwarts MJ. Diminished central activation during maximal voluntary contraction in chronic fatigue syndrome. Clin Neurophysiol 2004; 115: 2518-2524.

23. Schillings ML. Fatigue in neuromuscular disorders and chronic fatigue syndrome, a neurophysiological approach. Thesis 2005.
24. Duchateau J, Hainaut K. Electrical and mechanical changes in immobilized human muscle. J Appl Physiol 1987; 62: 2168-2173.
25. Voermans NC, Knoop H, Bleijenberg G, Engelen BG. Fatigue is associated with muscle weakness in Ehlers-Danlos syndrome: an explorative study . Physiotherapy 2011; 97: 170-4.

PART III

Neuromuscular features of Marfan syndrome

Clinical evaluation of Marfan syndrome patients

CHAPTER 11

Neuromuscular features in Marfan syndrome

Adapted from
Voermans N, Timmermans J, van Alfen N, Pillen S, op den Akker J, Lammens M, Zwarts MJ, van Rooij IA, Hamel BC, van Engelen BG.
Clin Genet. 2009;76:25-37.

Abstract

Marfan syndrome is a clinically and allelic heterogeneous, inherited connective tissue disorder with infrequently reported neuromuscular features. This study is the first to delineate these symptoms in a non-selected population.

Neuromuscular involvement was evaluated in ten Marfan patients through a standardized questionnaire, physical examination, nerve conduction study, needle electromyography, muscle ultrasound, laboratory investigation, and muscle biopsy. Existing neuroimages were screened for dural ectasia and spinal meningeal cysts. Twenty healthy controls with similar age distribution completed the questionnaire.

Various neuromuscular symptoms indeed occurred more frequently in the patients. Four older patients reported muscle weakness, five patients a mild-to-moderate reduction of vibration sense, and all older patients mentioned mild functional impairments. Nerve conduction studies showed axonal polyneuropathy in four and electromyography myopathic and neurogenic changes in all patients. Increased echo intensity and atrophy on muscle ultrasound was found in more than half of the patients. Muscle biopsies obtained in two patients showed myopathic changes in the older, female patient. Lumbosacral dural ectasia in combination with one or more lumbosacral spinal meningeal cysts was confirmed in seven patients, with one patient showing an additional thoracolumbar kyphoscoliosis.

The majority of Marfan patients displayed neuromuscular symptoms characterized as myopathy or polyneuropathy or both, and signs of lumbosacral radiculopathy, with symptoms being most pronounced in the older patients. Although meriting corroboration, these findings indicate a need to further the awareness of neuromuscular involvement in this population.

Introduction

Marfan syndrome is an inherited connective tissue disorder characterized by a varying pattern of organ involvement including the cardiovascular and pulmonary systems, eyes, skeleton, skin, and dura. At least 90% of all Marfan patients fulfilling the clinical diagnostic criteria show a mutation in the fibrillin-1 gene (*FBN1*) on chromosome 15, with 27% of the mutations being spontaneous.[1] Many symptoms do not present until puberty or later, and severe complications rarely develop before adulthood. In 1972, before the availability of elective open-heart surgery, Marfan patients tended to die from acute aortic dissection or rupture and had an average life expectancy of 32 (± 16) years. In 1993 the average life expectancy had been extended to 41 (± 18) years through management by expert centres.[2] With increasing life expectancy, the life-threatening cardiovascular involvement shifted to more chronic ocular, orthopaedic, and, possibly, neuromuscular complications.[3-7]

Although Marfan himself considered muscle involvement integral to his syndrome, neuromuscular features have since received little notice.[8] Muscle hypoplasia and myopathy were repeatedly reported, but, over the years, myopathy disappeared from the nosology and was no longer included in the diagnostic criteria.[9] Only recently has muscle involvement in Marfan syndrome received renewed attention, probably due to the increasing insight into the role of extracellular matrix molecules in muscle pathophysiology.[10,11] Investigating a family with Marfan syndrome and muscle weakness associated with respiratory failure, Behan et al. found that muscle biopsies showed fibrillin to be truncated and abnormally distributed in the endo- and perimysium.[12] A study of young female Marfan patients reported reduced quadriceps strength that could not fully be explained by a decrease in lean leg mass, major cardiovascular disease, or use of selective β-blockers.[13] This suggested qualitative skeletal-muscle alterations related to fibrillin abnormalities in the connective tissue of muscles.[13] In most Marfan patients growth and exercise does not lead to an increase in muscle mass, while patients with an early onset, severe, and rapidly progressive type have profound muscle hypoplasia and hypotonia.[13,14] The muscular features are probably related to fibrillin deficiency, which causes excess availability and signalling of transforming growth factor β, leading to impaired muscle-cell development and response to injury or inflammation.[14,15]

In addition to muscle involvement, radiculopathy caused by lumbosacral dural ectasia with spinal meningeal cysts may contribute to weakness and atrophy in Marfan syndrome.[16] Dural ectasia is probably caused by a weakening of the connective tissue of the dural sac due to fibrillin-1 deficiency and its severity seems to be related to age.[16] Symptoms that may occur are postural headaches, leg pain, abdominal pain, and low back pain.[17] Hydrostatic pressure, transmitted pulsation of the cerebrospinal fluid, and weakened dura may contribute to a gradual increase of the size of the spinal meningeal cysts, which, in turn, may cause lumbosacral root compression.[16-21]

To investigate whether and to which extent neuromuscular features occur in Marfan patients, and to determine whether they are related to age, we conducted a cross-sectional, observational study in ten non-selected, younger and middle-aged Marfan patients. We applied diagnostic procedures conventionally used in the work-up of neuromuscular diseases. To our knowledge, this is the first study to do so.

Methods

Study population

Without knowledge of their neuromuscular status, we invited 12 consecutive patients visiting our Marfan outpatient clinic to participate in our study. Two patients declined participation because they considered the study too time consuming. The 10 consenting patients (4 women; median age 40; age range 27 - 65) all fulfilled the diagnostic criteria of Marfan syndrome at that time (Ghent nosology, 1996).[4,9] Mutation of the fibrillin-1 gene (*FBN1*) was confirmed by DHPLC in nine patients, which is in agreement with the rate of mutation detection reported in the literature.[1]

To allow us to compare the self-reported neuromuscular symptoms of the Marfan group with those of the general population, we used the scores of 20 healthy controls (mostly hospital staff) who had completed the relevant questionnaire in an earlier study into neuromuscular involvement in Ehlers-Danlos syndrome.[22] The control group had a similar age distribution (median age 35; age range 22-64) but did contain proportionally but not significantly more female patients (n = 14).

The study was performed in accordance with the Helsinki criteria after approval by the local ethics committee. All participating patients gave their written informed consent prior to their participation.

Clinical studies

Standardized questionnaire

All patients and controls completed a brief, standardized questionnaire assessing muscle weakness, muscle hypotonia, frequent or continuous myalgia, and relative fatigability (compared to healthy age peers), while wheelchair use, use of walking aids, walking distance (with aids; < or 5 km), and engagement in sports (none or at least once weekly) were also charted. Other neurological symptoms and previous neurological diagnoses were noted if present.

Standardized physical examination

The patients were all examined by the same neurologist following a standardized protocol.

The clinical hallmarks of Marfan syndrome were noted, cranial nerves tested, and muscle mass qualitatively evaluated (normal vs. reduced) based upon the clinical experience of the investigator. The muscle strength of two trunk-muscle groups and 13 proximal and distal limb-muscle groups were tested manually and graded according to the Medical Research Council system (MRC score range 0 - 5 per muscle), resulting in a maximum MRC sum score of 140.[23] To verify the manual findings, muscle force was bilaterally quantified (in Newton) using a hand-held dynamometer (www.citec.nu) in five muscle groups, i.e., during shoulder abduction, elbow flexion and extension, a three-point grip, and knee extension, all performed in standardized positions. Per muscle group three measurements were taken and the mean force across measurements calculated, while for each patient the number of muscles in which this force was below the P5 value found in normal populations was noted.[24] The median force values for the male and female patients were then compared with the P5 and P50 values of the norm population. Coordination was tested bilaterally by use of a finger-nose-finger, a heel-to-knee-to-toe, and a tandem gait test. Sensation was examined by assessing vibration (with Rydell-Syffer tuning fork),[25] pain, and touch sense in the legs and arms, both proximally and distally; movement sense was tested in the distal legs and arms on a 3-point scale (0 = absent; 1 = reduced, and 2 = normal). Reflexes were evaluated bilaterally using the same scale (total score: 0 – 16), with scores < 16 indicating hyporeflexia. Functional measurements included the Vignos scale (assessing lower extremity functions with 1 signifying the ability to walk and climb stairs without assistance and 10 being confined to bed),[26] Brooke's scale (measuring upper-extremity functions; 1 = being able to fully abduct one's arms; 6 = no useful function of hands),[27] the Rivermead Mobility Index (evaluating disability related to bodily mobility; minimum score = 0; maximum score = 15 with higher scores reflecting better mobility), and the Modified Rankin Scale (gauging the degree of disability or dependence in daily activities; minimum score = 0; maximum score = 6, with higher scores reflecting more impairment).[28-30]

Ancillary investigations

Nerve conduction studies

By electrically stimulating peripheral nerves, nerve conduction studies (NCS) measure the conduction of motor and sensory nerves and together with needle electromyography (EMG; see below) the technique helps determine the presence of polyneuropathy and radiculopathy. Motor NCS in terms of distal motor latency, compound muscle-action potential (CMAP) amplitudes, and nerve conduction velocities (NCVs) were performed of the left peroneal nerve to the extensor digitorum brevis and anterior tibial muscles, the tibial nerve to the abductor hallucis muscles, and the median nerve to the abductor pollicis brevis muscle. Sensory NCS recording sensory-nerve action-potential (SNAP) amplitudes and NCVs were done of both sural nerves. H-reflexes of both tibial nerves to the m. soleus were

recorded. In case of a suspected polyneuropathy, NCS were supplemented with the ulnar nerve to the m. abductor digiti minimi and the superficial radial sensory nerve. The data of the clinical neurophysiological studies (i.e., our NCS and needle EMGs) were compared with the results in our database obtained in healthy controls. Polyneuropathy was defined in accordance with the guidelines of the American Association of Electrodiagnostic Medicine.[31]

Needle electromyography

By capturing the electrical potentials spontaneously generated by muscle cells at rest and during contraction, needle EMG allows the differentiation of neurogenic and myopathic diseases. We tested the anterior tibial, rectus femoris, biceps brachii, deltoid, and paraspinal L4 - L5 muscles, all on the left side. Motor unit action potentials (MUAPs) with a duration of 3 - 8 ms, polyphasic units, and normal-to-fast recruitment with low (< 1 mV) amplitudes on maximal contraction were classified as *'myogenic'*, while those with a 8 - 12 ms duration, an interference pattern, normal recruitment, and an amplitude of 1 - 2 mV on maximal contraction) were considered *'normal'*. MUAPs lasting in excess of 12 ms, having a poor-to-moderate recruitment with high amplitudes (> 2 mV) on maximal contraction were defined as *'neurogenic'*. Occurrence of myopathic and neurogenic units in arm and leg muscles was noted. To quantify suspected neurogenic or myopathic MUAPs, turns and amplitude analysis of the rectus femoris and biceps brachii muscle were performed when the routine needle examinations were suggestive of myopathic motor units.[32] The results of the clinical neurophysiological studies were reviewed by two neurologists.

Muscle ultrasound

Muscle ultrasound non-invasively visualizes muscle atrophy (reduced diameter) and intramuscular fibrosis and fatty infiltration (increased echo intensity), both of which occur in various neuromuscular disorders.[33] Examinations were performed bilaterally in five muscles (biceps brachii muscle, extensors and flexors in forearm, quadriceps femoris and anterior tibial muscle) using a standard technique previously described.[34,35] The mean muscle-echo intensities (i.e., the mean grey values of the muscles) were determined using a computer-assisted grey-scale analysis with 256 grey levels.[34] In the quadriceps femoris muscle, the rectus femoris was chosen for quantitative analysis of the echo intensity, as in severe neuromuscular disorders the outline of the vastus intermedius muscle can be difficult to define. Muscle thickness was measured with electronic callipers.

Echo intensity and muscle thickness are different for each muscle group and depend on age and sex.[35,36] Normal values for each muscle corrected for these variables were calculated with use of a previously established database.[36] To allow comparison of individual patients and muscles, the echo-intensity and muscle-thickness data were transformed into Z-scores, which reflect the number of standard deviations a measure deviates from normal, given a

certain age and sex.[35,36] Echo intensity was defined as abnormal when at least one muscle had a Z-score > 2, which cut-off value (Z-score of diameter < -2) was also used for muscle atrophy. The muscle-ultrasound readings and analyses were reviewed by one of our team's physicians Below, we will report cases with an increased echo intensity and atrophy, and detail the number of muscles in which both parameters were abnormal (Z-scores of echo intensity > 2 and Z-scores of diameter < -2) as well as their mean Z-scores.

Lumbosacral imaging

Because our NCS and EMGs uncovered lumbosacral radiculopathy, an experienced radiologist reviewed the existing MRI and CT studies (previously performed as screening for aortic dilatation) and looked for dural ectasia and spinal meningeal cysts at lumbosacral level.

Laboratory investigation

Creatine kinase (CK) was measured as a marker of muscular dystrophy (normal values: CK < 220 U/l (men) and CK < 170 U/l (women)). If the NCS and EMGs showed signs of polyneuropathy, laboratory tests were performed to exclude common causes of polyneuropathy (i.e., diabetes, thyroid dysfunction, diabetes, liver dysfunction, and vitamin B1 or B12 deficiency).

Muscle biopsies

Needle biopsy specimens were obtained from the right lateral vastus muscle in two patients that did not use oral anticoagulation and had given their informed consent for the procedure. Frozen sections of 10 µm were examined by the neuropathologist under a light microscope with Hematoxylin-Phloxine (HE), Periodic acid-Schiff, Sudan Black B, Trichrome-Gomori and enzyme histochemical staining (ATPase at pH 4.2, 4.6 and 10.3), succinic dehydrogenase, reduced nicotinamide adenine dinucleotide-tetrazolium reductase, cytochrome C oxidase, and acid phosphatise (NADH).

Definition of neuromuscular involvement

When a patient's outcomes on the questionnaire and/or physical examination were consistently abnormal and supported by abnormal results of relevant ancillary investigations, none of which could be explained by other common causes, we took this to be indicative of neuromuscular involvement.

Statistics

Statistical analysis was performed using SPSS version 14.0 (SPSS Inc, Chicago, IL, USA). Because of the small sample size, Fisher's exact test was used with dichotomous variables. Statistical significance was set at $P < 0.05$. A Pearson correlation coefficient was calculated to detect

correlations between age and muscle strength (MRC sum score) and impairment (Modified Rankin Scale, Brooke's scale, Vignos scale, and Rivermead Mobility Index), and the presence of neurogenic units at the lumbosacral level on electromyography. Subsequently, linear regression analyses were performed to quantify the relationships between age and the above-mentioned outcomes.

Results

Study population

Table 1 lists the characteristics of the study population.

Clinical studies

The results for the standardized questionnaire, physical examinations, and ancillary investigations are summarized in *Tables 2A, 2B,* and *3.*

Standardized questionnaires

The patients' neurological history revealed tension-type headache, migraine, temporal-lobe epilepsy, benign cramp-fasciculation syndrome, and cerebral abscesses following infectious endocarditis. Six patients used oral anticoagulants and nine a β-blocker.

Relative to their healthy peers, the patients more often reported muscle weakness (n = 4), muscle hypotonia (n = 2), myalgia (n = 9), easy fatigability (n = 8), and walking distance < 5 km (due to pain and muscle weakness; n = 4) and they also tended to engage less in sports. One patient used a walking stick (F65), and one knee braces and a rolling walker (F58). Overall, the proportion of neuromuscular complaints was larger in the older patients. One patient reported previous lower back pain radiating to both legs (M32) and three older patients reduced sensation in the legs and a feeling of insecurity when walking (F58, M59, and F65). One older patient (F58) reported faecal and urinary retention requiring intermittent bladder catheterisation and manual removal of faeces. No complaints of (nocturnal) respiratory failure were reported.

Standardized physical examination

Various typical, clinical hallmarks of Marfan syndrome were observed in all patients, i.e., positive wrist and thumb signs due to arachnodactyly, long arms with elbow contractures, joint hypermobility, increased arm span-to-height ratios, high-arched palate, (kypho)scoliosis, pectus carinatum or excavatum, and ectopia lentis. Ptosis was observed in two patients, one of whom also showed drooping of both lower eyelids (*Figure 1*). No other cranial nerve abnormalities were found. The four oldest patients had mild-to-moderate muscle weakness

Table 1 The genetic test results, histories, and medication use for all ten Marfan patients.

Sex and age	*FBN1* mutation (tested with DHPLC)	Medical history	Neurological history	Medication
M 27	Missense mutation exon 26 (3302A>G; Y1101C)	Endocarditis lenta; ascending aorta dilatation: Bentall procedure (at age 12); mitral valve prolaps: prothesis		sotalol, oral anticoagulation
M 28.1	Base insertion (frame shift mutation) exon 23 (2851insG)	VSD defect, surgically corrected; aortic root dilatation: Bentall procedure (at age 12)	Temporal lobe epilepsy; migraine	carbamazepine, metoprolol, oral anticoagulation
M 28.2	Base substitution in nitro 46 (IVS46+5G>A) resulting in skipping of exon of *FBN* mRNA	Lens luxation; ascending aorta dilatation and aortic insufficiency: Bentall procedure (at age 14)		metoprolol, oral anticoagulation
M 32	Missense mutation exon 63 (7916A>G ;Y2639C)	Lens luxation; mitral prolaps and insufficiency both surgically corrected (at age 31)		metoprolol, lisinopril
F 33	Missense mutation exon 56 (6883T>C; C2295R)	Inguinal hernia; hormonal growth inhibition; mitral valve prolaps; scoliosis: brace		metoprolol
M 46	Although DHPLC analysis did not reveal a mutation, this patient did fulfil the diagnostic criteria (meeting major criteria for skeletal, ocular, and cardiovascular systems, and dura)	Inguinal hernia; pectus carinatum; lens luxation; retinal detachment; aortic root and ascending aorta dilatation, both surgically corrected (at age 42); testis carcinoma	Hypophysis adenoma; tension-type headache	metoprolol, simvastatin, carbasalaatcalcium, thyrax, cabergoline
F 55	Missense mutation exon 59 (7364G>A; C2445Y)	Aortic dissection: Bentall procedure (at age 43); endocarditis after dental treatment	Benign cramp-fasciculation syndrome; cerebral abscesses	metoprolol, oral anticoagulation, oral anticoagulation
F 58	Missense mutation exon 6 (640G>C; G214R)	Lens luxation; thoracolumbar kyphoscoliosis: spondylodesis Th10-L2; mild dilatation of aortic root; mitral valve prolaps	Lumbar root compression with paresis and sensation disturbances	timoptol
M 59	Missense mutation in exon 55(6827G>A; C2276Y)	Aortic dissection; Bentall procedure (at age 55) lens luxation	Tension-type headache	atenolol, carbasalaatcalcium, oral anticoagulation
F 65	Genomic deletion exon 50 - 63	Ascending aorta dilatation and aorta insufficiency: Bentall procedure (at age 47); descending aorta dissection, surgically corrected		propanolol, simvastatin, oral anticoagulation

DHPLC: Denaturing high performance liquid chromatography. *FBN-1*: Fibrillin-1 gene.

Table 2A Self-report outcomes for the patients and the healthy controls and patients' results for the physical examination.
The numbers (percentages) of the patients and healthy controls reporting the symptom are indicated with the significance levels reflecting the between-group differences in the frequency of the subjective symptoms; + indicates the actual patients reporting and/or showing the symptoms upon examination and their mean scores per test/scale are listed. Absence of subjective symptoms or normal results on physical examination are specified as '-'.

	Marfan patients (n=10)	Healthy controls (n=20)	Patients vs. healthy controls P-value (#)	M 27	M 28.1	M 28.2	M 32	F 33	M 46	F 55	F 58	M 59	F 65
Standardized questionnaire													
Muscle weakness	4	0	P = 0.008	-	-	-	-	-	-	+	+	+	+
Muscle hypotonia	2	0	n.s.	-	-	-	-	-	-	+	+	-	-
Myalgia (frequent or continuous)	9 (90%)	2 (10%)	P < 0.001	-	+	+	+	+	+	+	+	+	+
Easy fatigability (relative to healthy age peers)	8 (80%)	0	P < 0.001	+	+	+	-	-	+	+	+	+	+
Walking distance < 5 km	4 (40%)	1 (5%)	P = 0.031	-	-	-	-	-	+	-	+	+	+
Use of walking aids	2 (20%)	0	n.s.	-	-	-	-	-	-	-	+	-	+
Wheelchair use	1 (10%)	0	n.s.	-	-	-	-	-	-	-	+	-	-
Engagement in sports (at least once a week)	4 (40%)	14 (70%)	n.s.	+			+	+		+			
Physical examination													
Mean MRC sum score (SD) (0-140)	133 (13.2)			140	140	140	140	140	140	131	101	138	118
Number of muscles with reduced strength on dynamometry				4	0	1	0	2	2	7	2	4	7
Reduced muscle mass	4			+	-	-	-	-	+	-	-	+	+
Reduced coordination (coordination sum score range 0-10)	4			-	-	-	-	-	+ (6)	-	+ (4)	+ (6)	+ (4)

Reduced sensation (sensation sum score range 0-60)	5	-	-	-	-	-	+ (46)	+ (58)	+ (56)	+ (53)	+ (56)
Hyporeflexia (Total reflex score range 0-16)	3	-	-	-	-	-	+ (14)	-	+ (8)	-	+ (12)
Mean Vignos score (1-10)	2.1	1	1	1	1	1	2	2	6	3	3
Mean Brooke score (1-6)	1.1	1	1	1	1	1	1	1	1	1	2
Mean Rivermead score (0-15)	13.6	15	15	15	15	15	15	14	7	13	12
Mean Modified Rankin scale (0-6)	1.4	0	0	2	0	0	2	2	4	1	3

n.s.: no statistically significant between-group difference. (#): Fisher's exact test. MRC: Medical Research Council.

Table 2B Dynamometry results of the group of Marfan patients.
The 2nd and 5th columns list the sex-related median muscle forces (P50) for the five muscle as measured with a hand-held dynamometry for the patients and the 3rd, 4th, 6th and 7th columns the sex-related P5 and P50 values for the healthy controls.

	Men			**Women**		
	Marfan patients (n=6)	**Healthy controls[24]**		**Marfan patients (n=4)**	**Healthy controls[24]**	
	P50 force (N) (range)	*P5 force (N)*	*P50 force (N)*	*P50 force (N) (range)*	*P5 force (N)*	*P50 force (N)*
Shoulder abduction	131 (109 - 163)	111	160	78 (25 - 140)	75	105
Elbow flexion	183 (66 - 240)	216	> 250	122 (86 - 149)	146	190
Elbow extension	131 (106 - 151)	115	156	90 (72 - 121)	80	105
Three point grip	122 (66 - 135)	94	125	71 (45 - 93)	65	86
Knee extension	205 (143 - 238)	> 160	> 160	120 (72 - 147)	> 160	> 160

Figure 1 Various observations in three Marfan patients.

A: Generalized muscle hypoplasia in a 27-year-old male Marfan patient who also showed kyphoscoliosis, pectus carinatum, elbow contractures, and arachnodactyly. **B**: Bilateral ptosis in a 28-year-old male Marfan patient, with bilateral drooping of the lower eyelids showing the whites of the eyes between the eyelid and the iris. **C:** Asymmetric atrophy and dystrophic changes in the right lower and upper leg of a 58-year-old female Marfan patient.

(MRC sum scores < 140). The dynamometry results corroborated the findings obtained in the manual tests: all patients with reduced MRC sum scores had at least two muscles with force values below the normal P5 (*Table 2A*). Furthermore, the median force during elbow flexion in the men and during shoulder abduction, elbow flexion, and knee extension in the women was below or near the normal P5 value (*Table 2B*). Muscle weakness correlated with age ($r = -0{,}67$; $P = 0.04$), with a 5.8 decrease of the MRC sum score every 10 years ($P = 0.04$). Muscle mass was reduced in four patients, with the two male patients reporting that it had overall been poor since childhood (i.e., generalized muscle hypoplasia; M27, M46). The two female patients stated that the muscle mass in their legs had gradually diminished in the past few years (i.e., muscle atrophy; F58, F65; also see *Figure 1*). Coordination was reduced in four and sensation in five (mostly involving mild reductions in vibration sense in the legs and/or arms).

Hyporeflexia occurred in three patients and consisted of reduced ankle-jerk reflexes (M46, F65) and absent knee-jerk and ankle-jerk reflexes (F59), which findings are consistent with the occurrence of polyneuropathy and radiculopathy in these patients.

The functional assessment revealed mild impairments in the older patients (M46, M59, F55, F58, and F65): with a mean score of 2.1/10 the Vignos lower-extremity score was increased in half of the patients. Brooke's upper-extremity score was abnormal in one patient (mean score: 1.1/6). The Rivermead Mobility Index was reduced in four patients yielding a mean index of 13.6/15. The Modified Rankin Scale showed abnormal scores in six patients (mean score: 1.4/6), all indicating mild disability. Impairments gradually increased with age, with all outcomes except the Brooke scores showing significant correlations: Vignos $r = 0.76$; $P = 0.01$ with a 0.8-point increase each decade ($P = 0.01$); Rivermead Mobility Index $r = -0.66$; $P = 0.04$ with a 1.1-point decrease ($P = 0.04$), and the Modified Rankin Scale $r = 0.73$; $P = 0.02$ with a 0.7-point increase ($P = 0.02$).

Ancillary investigations

Nerve conduction data

NCS results were abnormal in five patients. In four patients findings were compatible with an axonal sensorimotor polyneuropathy, of whom three, as well as another patient, additionally showed signs of a lumbosacral radiculopathy (CMAP reduction > SNAP reduction; increased H-reflex latency), with involvement of the L5 level in all four, and also L3-L4 level in two patients.

Electromyographic readings

The needle EMGs were abnormal in all patients, revealing a mixed pattern with small and enlarged MUAPs. The younger patients overall had myopathic MUAPs in both arms and legs, pointing to myopathy. The older patients predominantly had neurogenic MUAPs mainly in their legs and paravertebral muscles, indicating lumbosacral radiculopathy, compatible with the NCS findings. The presence of neurogenic units in the EMGs of the lumbosacral muscles correlated with age ($r = 0.70$; $P = 0.03$), showing an increase of 12% over 10 years ($P = 0.03$). A mild myopathic shift was seen in the turns and amplitude analysis of the rectus femoris muscle in two of the seven patients tested and in the biceps brachii muscle in 3/9, outcomes that are indicative of myopathy.

Myopathic units typically have short durations and small amplitudes accompanied by polyphasia. In case of denervation due to axonal polyneuropathy or radiculopathy with subsequent reinnervation, the units become larger (lasting longer at higher amplitudes) and these superimposed neurogenic changes may mask any myopathic changes, which may explain why in the older patients myogenic features were no longer found.

Table 3 Results of the ancillary investigations.
The second column lists the number of patients in which mentioned tests revealed abnormal results, the number of muscles in which increased echo intensity and reduced muscle diameter was found, and the mean Z-score of echo intensity and muscle diameter for each patient. In the other columns '+' indicates the patients showing abnormal test results. Normal results are specified as '-'.

	Patient (n=10)	**M 27**	**M 28.1**	**M 28.2**	**M 32**	**F 33**	**M 46**	**F 55**	**F 58**	**M 59**	**F 65**
Laboratory investigations											
Median creatine kinase (U/l) (range)	100 (63 - 225)	217	225	124	99	71	63	70	126	70	100
Nerve conduction											
Abnormal	5	-	-	-	+	-	+	-	+	+	+
Axonal polyneuropathy	4	-	-	-	-	-	+	-	+	+	+
Signs of radiculopathy (at least one lumbosacral level)	4	-	-	-	+	-	+	-	+	-	+
Electromyography											
Abnormal	10	+	+	+	+	+	+	+	+	+	+
Mixed pattern of small and enlarged MUAPs:											
myogenic = neurogenic	2	-	+	-	-	-	+	-	-	-	-
myogenic > neurogenic	5	+	-	+	+	+	-	+	-	-	-
myogenic < neurogenic	3	-	-	-	-	-	-	-	+	+	+
Mild myopathic pattern in T/A of rectus femoris	2 (n = 7)	+	-	-	n.p.	-	-	+	n.p.	n.p.	-
Mild myopathic pattern in T/A of biceps brachii	3 (n = 9)	+	+	-	-	n.p.	-	+	-	-	-
Muscle ultrasound											
Increased Echo intensity	6	+	-	-	-	-	+	+	+	+	+
Number of muscles with increased echo intensity (0 - 10)		1	0	0	0	0	6	1	3	1	1
Mean Z-score of echo intensity		0.8	0.0	1.1	0.5	0.8	1.9	0.6	1.6	0.9	1.1

Muscle atrophy	8	+	-	+	-	+	+	+	+	+	+
Number of muscles with muscle atrophy (0 - 10)		6	0	1	0	1	7	2	3	2	1
Mean Z-score of muscle diameter		- 3.4	0.0	- 0.5	0.0	- 1.2	- 2.1	- 1.1	- 0.6	- 0.8	- 0.8
Muscle biopsy (n = 2)											
Myopathic features	1	n.p.	n.p.	n.p.	-	n.p.	n.p.	n.p.	+	n.p.	n.p.
MRI (screening for aortic dilatation)											
Presence of dural ectasia	7	+	+	+	-	-	+	+	+	-	+
Presence of meningeal spinal cysts	8	+	+	+	-	+*	+	+	+	-	+

CMAP: Compound muscle action potentials. SNAP: Sensory nerve action potential. MUAP: Motor unit action potential. CRD: Complex repetitive discharges. T/A: Turns and amplitude analysis. *: Indicates the presence of a single dural cyst at the left thoracolumbal spine but not at the lumbosacral level. n.p.: Not performed.

Ultrasound findings

Muscle ultrasound data were obtained in all 10 patients and showed increased echo intensities in six. The elevations were most pronounced in the tibial anterior muscles and in the forearm extensors and flexors (*data not shown*). Eight patients showed muscle atrophy, which was most pronounced in the forearm extensor and the quadriceps muscles (*data not shown*).

Lumbosacral imaging data

For nine patients recent MRI scans were available, with additional CT scans (both conducted to screen for aortic dilatation) for three of these patients; for one patient only a CT scan had been performed. For one patient lumbosacral MRI images from a previous examination because of weakness and atrophy in her legs were also available (F58).

Lumbosacral dural ectasia in combination with one or more lumbosacral spinal meningeal cysts was confirmed in seven patients, with one patient showing an additional thoracolumbar kyphoscoliosis (F58). One of the three patients without lumbosacral dural ectasia had a left thoracolumbar cyst but no lumbosacral cysts (F33), and the two other patients had neither lumbosacral dural ectasia nor cysts. Because the MRI scans were originally optimized for routine aortic-diameter monitoring only, the quality of the images showing the structures in the lumbosacral region was suboptimal, thus preventing a detailed evaluation of the relationship between meningeal spinal cysts and radices.

Laboratory test results

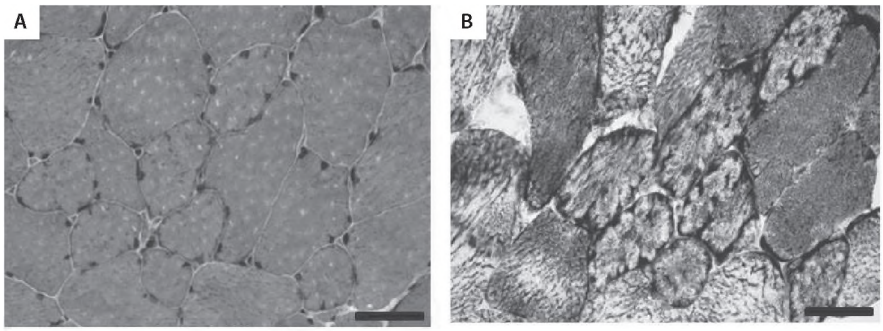
Creatine kinase was normal in nine patients and borderline in one. In the four patients with a polyneuropathy the results of the ancillary lab tests were normal (*data not shown*).

Muscle biopsies

Enzymehistochemical staining excluded the presence of common congenital myopathies or dystrophies in the muscle biopsies of both patients tested, and there were no signs of inflammation. The biopsy of one patient (M32) was normal while the other patient's specimen (F58) revealed myopathic features, which consisted of a mild increase of muscle-fibre calibre variation and scattered lobulated fibres *(Figure 2)*. The lobular or trabecular pattern of the oxidative enzyme reaction in these lobulated fibres reflects an abnormal distribution of the intermyofibrillar mitochondria, possibly caused by a malfunctioning putative anchoring mechanism. Lobulated fibres can occur as a nonspecific alteration of muscle fibres in many diverse, often longstanding, myopathies such as facioscapulohumeral muscular dystrophy, limb-girdle muscular dystrophies, and hypothyroidism.[37]

Figure 2 Muscle biopsy of the left quadriceps muscle of a 58-year-old female Marfan patient revealing myopathic features.

A: Increased variation in the muscle fibre diameter (HE). **B**: Several lobulated fibres are visible in the centre of the picture (NADH). Bar = 50 micrometer.



Discussion

Our comprehensive investigations demonstrated neuromuscular involvement in the majority of the ten clinically and genetically proven Marfan patients we tested, albeit to a variable degree. Symptoms included mild-to-moderate myopathy and polyneuropathy and occasional signs of lumbosacral radiculopathy. The deficits were most pronounced in the older patients, with muscle force, functional impairment, and neurogenic units on electromyography increasing with age. Our findings underscore the need to inform medical practitioners that the range of complications that can occur in patients with Marfan syndrome extends from cardiovascular, ocular, and orthopaedic symptoms to neuromuscular involvement and lumbosacral radiculopathy.

Previous cardiovascular complications (endocarditis, mitral-valve prolaps, and aortic root/ascending aorta dilatation) and the medication used (β-blocker) may have contributed to the fatigue reported by our Marfan patients and may hence be common in all patients. Furthermore, simvastatin can cause myalgia with signs of myopathy and increases the risk of polyneuropathy.[38] However, results of previous studies suggest that cardiovascular disease and side effects of medication cannot fully explain the muscle weakness in Marfan patients.[7,13] Likewise, results from our study suggest that Marfan syndrome is directly associated with moderate-to-mild myopathy and/or polyneuropathy, while the presence of spinal meningeal cysts was related to lumbosacral radiculopathy.

Myopathy in Marfan syndrome might be linked to abnormal fibrillin in muscle connective tissue (endo-, peri- and epimysium),[12,39] which results in abnormal muscle-cell development and reactions to injury or inflammation.[14,15] Abnormalities in muscle connective tissue further influence force transmission between muscle cells and their environment, thus also contributing to muscle weakness.[40,41]

In addition to a myopathy, four patients had an axonal polyneuropathy. Although fibrillin-1 and fibrillin-2 have been found in the connective tissue sheaths of peripheral nerves,[42,43] peripheral neuropathy in Marfan syndrome has only been reported once.[44] Abnormal packing of peripheral nerves due to fibrillin deficiency may increase the susceptibility to pressure or stretch, as in other inherited connective tissue disorders.[45] To establish whether fibrillin abnormalities also affect axonal functioning, clinical, electrophysiological, and histological examinations are warranted in larger cohorts of Marfan patients.

In most of the older patients physical examination and nerve conduction studies as well as electromyography uncovered signs of lumbosacral radiculopathy. This co-occurred with dural ectasia and spinal meningeal cysts. However, due to the limited resolution of lumbosacral structures, we were unable to determine the exact nature of the structural relationship between meningeal spinal cysts and radices based on the scans we had at our disposal, which requires targeted MRI imaging of the lumbosacral spine as well as a larger patient sample. Nevertheless, co-occurrence of clinical and electrophysiologically confirmed signs of radicular dysfunction and dural ectasia with meningeal cysts suggests a partial causal association between the two irregularities, which assumption is supported by previous reports.[19]

Our findings of neuromuscular involvement in Marfan syndrome confirm the results of recent questionnaire studies on muscle function in Marfan syndrome in relation to ageing,[7,12,13] and the findings reported in a mouse model of Marfan syndrome.[14] In analogy, we recently demonstrated that neuromuscular features also frequently occur in another inherited connective tissue disorder, i.e., Ehlers-Danlos syndrome.[22,41] A further comparison can be made with Ullrich congenital muscular dystrophy and Bethlem myopathy, both of which are caused by collagen VI deficiency.[46,47]

The small number of patients tested in this study is a limitation. It was our aim to perform an observational and in-depth investigation to confirm the findings of questionnaire studies, but this design came with some restrictions. Our findings and conclusions evidently warrant corroboration in larger samples and designs that would also allow correlations between age, the extent of the cardiovascular and neuromuscular involvement and the dural ectasia to be further delineated.

In short, the majority of Marfan patients examined in this study showed neuromuscular involvement, primarily consisting of myopathy and polyneuropathy, with some signs of (poly)radiculopathy. Overall, the symptoms were most pronounced in the older patients.

These results call for further research in larger groups, and, if confirmed, should lead to increased attention to neuromuscular symptoms and lumbosacral radiculopathy, especially since the life expectancy of patients with Marfan syndrome has increased.

Reference List

1. Loeys B, De Backer J, Van Acker P, Wettinck K, Pals G, Nuytinck L, Coucke P, De Paepe A. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. Hum Mutat 2004; 24: 140-146.
2. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, Boxer M, Devereux RB, Tsipouras P. Life expectancy in the Marfan syndrome. Am J Cardiol 1995; 75: 157-160.
3. von Kodolitsch Y, Robinson PN. Marfan syndrome: an update of genetics, medical and surgical management. Heart 2007; 93: 755-760.
4. Dean JC. Marfan syndrome: clinical diagnosis and management. Eur J Hum Genet 2007; 15: 724-733.
5. Jones KB, Sponseller PD, Erkula G, Sakai L, Ramirez F, Dietz HC, Kost-Byerly S, Bridwell KH, Sandell L. Symposium on the musculoskeletal aspects of Marfan syndrome: meeting report and state of the science. J Orthop Res 2007; 25: 413-422.
6. Ramirez F, Dietz HC. Marfan syndrome: from molecular pathogenesis to clinical treatment. Curr Opin Genet Dev 2007; 17: 252-258.
7. Hasan A, Poloniecki J, Child A. Ageing in Marfan syndrome. Int J Clin Pract 2007; 61: 1308-1320.
8. Marfan AB. Un cas de déformation congénitale des quatres membres, plus prononcée aux extrémités caractérisée par l'allongement des os avec un certain degrée d'amincissement. Bull Mem Soc Med Hop (Paris) 1896; 13: 220-226.
9. De Paepe AM, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996; 62: 417-426.
10. Lampe AK, Bushby KM. Collagen VI related muscle disorders. J Med Genet 2005; 42: 673-685.
11. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
12. Behan WM, Longman C, Petty RK, Comeglio P, Child AH, Boxer M, Foskett P, Harriman DG. Muscle fibrillin deficiency in Marfan's syndrome myopathy. J Neurol Neurosurg Psychiatry 2003; 74: 633-638.
13. Percheron G, Fayet G, Ningler T, Le Parc JM, Denot-Ledunois S, Leroy M, Raffestin B, Jondeau G. Muscle strength and body composition in adult women with Marfan syndrome. Rheumatology (Oxford) 2007; 46: 957-962.
14. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007; 13: 204-210.
15. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, Ramirez F, Sakai LY, Dietz HC. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet 2003; 33: 407-411.
16. Fattori R, Nienaber CA, Descovich B, Ambrosetto P, Reggiani LB, Pepe G, Kaufmann U, Negrini E, von Kodolitsch Y, Gensini GF. Importance of dural ectasia in phenotypic assessment of Marfan's syndrome. Lancet 1999; 354: 910-913.
17. Foran JR, Pyeritz RE, Dietz HC, Sponseller PD. Characterization of the symptoms associated with dural ectasia in the Marfan patient. Am J Med Genet A 2005; 134A: 58-65.
18. Di Lazzaro V, Pilato F, Dileone M, Minicuci G, Profice P, Colosimo C, Tartaglione T, Tonali PA. Extradural arachnoid cyst with lumbosacral cord and root compression in marfan syndrome. Arch Neurol 2007; 64: 284-285.
19. Hoshino Y, Edakuni H, Shimada H, Hayashi S, Machida M, Shimano S, Taya T, Ohki I, Takahashi A, Kurihara T, Yamada I, Arai T, Miyamoto Y, Togo Y. Sacral arachnoid cyst associated with marfan syndrome. Intern Med 2005; 44: 271-273.
20. Sponseller PD, Shindle M. Orthopedic problems in Marfan syndrome. In: Robinson PN, Godfrey M, editors. Marfan Syndrome: a primer for clinicians and scientists. New York: Kluwer Academic / Plenium Publishers; 2004. p. 24-34.
21. Nabors MW, Pait TG, Byrd EB, Karim NO, Davis DO, Kobrine AI, Rizzoli HV. Updated assessment and current classification of spinal meningeal cysts. J Neurosurg 1988; 68: 366-377.
22. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
23. Peterson-Kendall F, Kendall-McCreary E, Geise-Provance P, McIntyre-Rodgers M, Romani WA. Muscles testing and Function with Posture and Pain. Baltimore, MD, USA: Lipincott Williams & Wilkins; 2005.
24. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. J Neurol Neurosurg Psychiatry 1991; 54: 244-247.

25. Martina IS, van Koningsveld R, Schmitz PI, van der Meche FG, van Doorn PA. Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. European Inflammatory Neuropathy Cause and Treatment (INCAT) group. J Neurol Neurosurg Psychiatry 1998; 65: 743-747.
26. Thompson RA, Vignos PJ, Jr. Serum aldolase in muscle disease. AMA Arch Intern Med 1959; 103: 551-564.
27. Brooke MH, Griggs RC, Mendell JR, Fenichel GM, Shumate JB, Pellegrino RJ. Clinical trial in Duchenne dystrophy. I. The design of the protocol. Muscle Nerve 1981; 4: 186-197.
28. Collen FM, Wade DT, Robb GF, Bradshaw CM. The Rivermead Mobility Index: a further development of the Rivermead Motor Assessment. Int Disabil Stud 1991; 13: 50-54.
29. Rankin J. Cerebral vascular accidents in patients over the age of 60. III. Diagnosis and treatment. Scott Med J 1957; 2: 254-268.
30. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988; 19: 604-607.
31. England JD, Gronseth GS, Franklin G, Miller RG, Asbury AK, Carter GT, Cohen JA, Fisher MA, Howard JF, Kinsella LJ, Latov N, Lewis RA, Low PA, Sumner AJ. Distal symmetrical polyneuropathy: a definition for clinical research. A report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Arch Phys Med Rehabil 2005; 86: 167-174.
32. Fuglsang-Frederiksen A, Scheel U, Buchthal F. Diagnostic yield of the analysis of the pattern of electrical activity of muscle and of individual motor unit potentials in neurogenic involvement. J Neurol Neurosurg Psychiatry 1977; 40: 544-554.
33. Pillen S, Arts IM, Zwarts MJ. Muscle ultrasound in neuromuscular disorders. Muscle Nerve 2008; 37: 679-693.
34. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. Muscle Nerve 2003; 27: 693-698.
35. Pillen S, Verrips A, van Alfen N, Arts IM, Sie LT, Zwarts MJ. Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. Neuromuscul Disord 2007; 17: 509-516.
36. Arts IM, Pillen S, Overeem S, Schelhaas HJ, Zwarts MJ. Rise and fall of skeletal muscle size over the entire life span. J Am Geriatr Soc 2007; 55: 1150-1152.
37. Weller B, Carpenter S, Lochmuller H, Karpati G. Myopathy with trabecular muscle fibers. Neuromuscul Disord 1999; 9: 208-214.
38. Gaist D, Jeppesen U, Andersen M, García Rodríguez LA, Hallas J, Sindrup SH. Statins and risk of polyneuropathy: a case-control study. Neurology 2002; 58: 1333-1337.
39. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
40. Huijing PA. Epimuscular myofascial force transmission between antagonistic and synergistic muscles can explain movement limitation in spastic paresis. J Electromyogr Kinesiol 2007; 17: 708-24.
41. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
42. Isogai Z, Ono RN, Ushiro S, Keene DR, Chen Y, Mazzieri R, Charbonneau NL, Reinhardt DP, Rifkin DB, Sakai LY. Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. J Biol Chem 2003; 278: 2750-2757.
43. Charbonneau NL, Dzamba BJ, Ono RN, Keene DR, Corson GM, Reinhardt DP, Sakai LY. Fibrillins can co-assemble in fibrils, but fibrillin fibril composition displays cell-specific differences. J Biol Chem 2003; 278: 2740-2749.
44. Barrison IG, Isenberg DA, Kane SP. Arachnodactyly with unusual neuromyopathic and skeletal abnormalities. J R Soc Med 1980; 73: 64-68.
45. Voermans NC, Drost G, van Kampen A, Gabreels-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG. Recurrent neuropathy associated with Ehlers-Danlos syndrome. J Neurol 2006; 253: 670-671.
46. Camacho VO, Bertini E, Zhang RZ, Petrini S, Minosse C, Sabatelli P, Giusti B, Chu ML, Pepe G. Ullrich scleroatonic muscular dystrophy is caused by recessive mutations in collagen type VI. Proc Natl Acad Sci U S A 2001; 98: 7516-7521.

47. Jobsis GJ, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA. Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. Nat Genet 1996; 14: 113-115.

CHAPTER 12

Radicular dysfunction due to spinal deformities in Marfan syndrome at older age: three case reports

Adapted from
Voermans NC, Hosman AJ, van Alfen N, Bartels RH, de Kleuver M, op den Akker JW, van Engelen BG.
Eur J Med Genet. 2010;53:35-9.

Abstract

Marfan syndrome is an inherited connective tissue disorder due to mutations in fibrillin-1. It presents with cardiovascular, ocular, skeletal, pulmonary and dural signs and symptoms. Some of the symptoms of later onset are those associated with scoliosis and dural ectasia. This is the enlargement of the neural canal especially in the lower lumbar and sacral region and occurs in over 90% of Marfan patients.

We here report three patients with lumbar and/or sacral radiculopathy due to (kypho) scoliosis and dural ectasia with spinal meningeal cysts. The pain, muscle weakness, muscle atrophy, and sensory disturbances illustrate the severe neurological complications which may occur in Marfan syndrome, especially at later age. Awareness of these complications and development of management protocols is essential since life expectancy of Marfan patients has increased. Marfan syndrome might gradually become recognized as an inherited connective tissue disorder with potentially severe neurological complications during ageing.

Introduction

Marfan syndrome is an autosomal dominant inherited disorder of the connective tissue with an estimated prevalence of 1 in 5000. Phenotypic expression is variable and mutations in fibrillin-1 on chromosome 15 are detected in 66 - 91% of cases, of which 27% are de novo.[1] Clinical presentation includes cardiovascular, ocular, skeletal, dermal, pulmonary, and dural signs and symptoms. Many symptoms present during puberty or later and severe complications (e.g. severe scoliosis and pectus excavatum, spontaneous pneumothorax, retinal detachment or sight-threatening glaucoma resulting from a dislocated lens) rarely develop before adulthood. With the availability of elective cardiac surgery and management by expert centres, the mean life expectancy of Marfan patients has increased from 32 years in 1972 to 41 years in 1993, and probably even much further since then.[2-9]

This increased life expectancy may be accompanied by increase of spinal and neurological complications. Some of the symptoms of later onset are those associated with scoliosis and dural ectasia.[10] Scoliosis affects around 60% of Marfan patients and may rapidly progress during growth spurts, leading to marked deformity, back pain, and restricted ventilation.[4,11] Dural ectasia is the enlargement of the neural canal especially in the lower lumbar and sacral region, which occurs in over 90% of Marfan patients,[12] and less frequently in Ehlers-Danlos syndrome or neurofibromatosis type I.[13,14] It is probably caused by ongoing hydrostatic pressure and transmitted pulsation of cerebrospinal fluid, which progressively dilates the dural sac since the elastin composition of the dura mater is altered due to fibrillin deficiency.[5,12] It can present as thinning of the cortex of the vertebral bodies, pedicles, and laminae of the vertebrae, as widening of the neural foraminae, or as an anterior (sacral) meningocele.[10] The diameter of dural ectasia seems to be related to age, and dural ectasia can be present for a long time without producing any noticeable symptoms.[12,15] Symptoms can include leg pain, abdominal pain, lower back pain, or postural headaches.[11] When the dilated part of the dura protrudes through the neural foramina, arachnoid cysts may develop either outside or inside the dura, which are respectively referred to as extradural cysts: type I – II and intradural cysts: type III).[12,16-18] Since classification based on imaging studies is difficult, these arachnoid cysts might better be described as spinal meningeal cysts. They have been associated with additional neurological symptoms, including sphincter disturbance and radicular compression with neurological deficits, especially in the elder patients.[4,5,11,12,16,16,19-22] Studies on treatment of symptomatic dural ectasia with or without spinal meningeal cysts are not available.

We here report three patients with lumbar or sacral radiculopathy due to (kypho)scoliosis and dural ectasia with spinal meningeal cysts. The pain, muscle weakness, muscle atrophy, and sensory disturbances illustrate the severe neurological complications which may occur in Marfan syndrome, especially at a later age.

Case reports

The patients were referred to the neuromuscular outpatients department by the cardiologist at the multidisciplinary outpatients department for Marfan patients in one of the three specialized Marfan centres in the Netherlands Marfan syndrome was diagnosed based on the clinical diagnostic criteria[14] and confirmed by *FBN1* mutation analysis in all three patients. Patient 2 has previously been reported in our systematic study on neuromuscular features in ten Marfan syndrome patients (F59).[15]

Patient 1

This 64-year year-old male patient suffered from severe pain for four years, radiating from his lower back to his left upper leg during walking or standing upright. Lower back pain had been present for longer, but was never as severe. The current pain had a burning character with a Visual Analogous Score of 9/10 when upright, completely disappearing after lying down. Pain severity had gradually increased and the pain also radiated to his right leg since one year. He was severely limited in his daily activities since he could not stand or walk longer than ten minutes because of the severe pain. He had also noticed numbness of the ventral and medial side of his lower leg. Erections had disappeared ten years ago, and the patient suffered from urinary hesitation, which was ameliorated by tamsulosin. Defecation was normal. Transdermal opiod patches and gabapentin had only resulted in partial pain reduction.

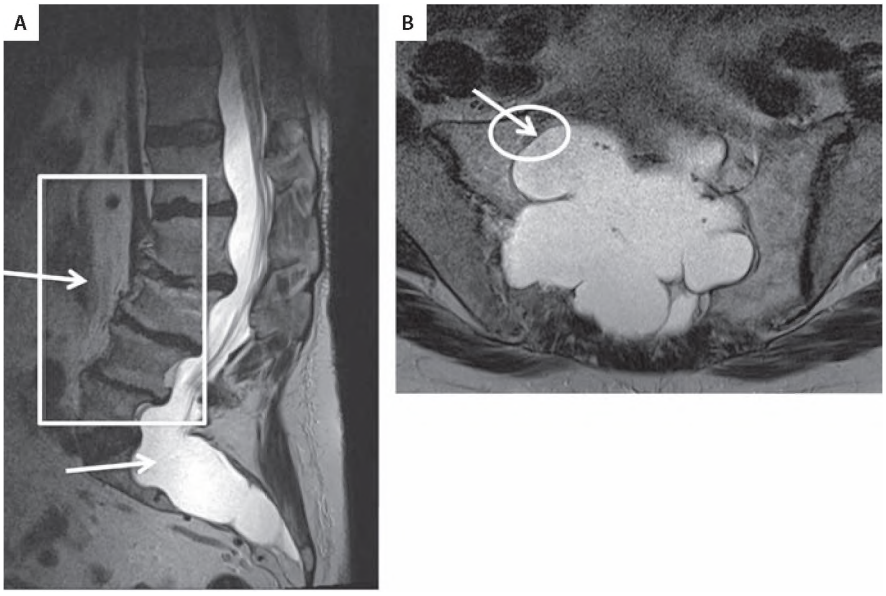
Physical examination showed typical signs of Marfan syndrome, with mild weakness of hip flexion and of the ankle dorsiflexors bilaterally (Medical Research Council (MRC) 4).[23] Vibration sense was reduced in both feet, and pain and touch sensation were reduced in dermatomes L2 - L4 on the left. Knee reflexes were normal, and ankle jerks were absent bilaterally.

Nerve conduction studies revealed an axonal polyneuropathy, with neurogenic changes in the tibialis anterior muscle on needle EMG examination. Neurogenic changes were also found in both rectus femoris muscles, with denervation in the L4 paraspinal muscles on both sides, indicating bilateral L4 radiculopathy. Neurogenic changes of an older date were also observed in the gastrocnemius muscle on the left side (right side not tested), compatible with previous S1 radiculopathy or axonal polyneuropathy. Sagittal MRI imaging of the lumbosacral spine revealed a kyphosis of segments L3 - L5, with extensive degenerative changes (irregular superior and inferior endplates, osteophytes, bulging discs). The body of L3 was mildly laterally displaced to the left in relation to the body of L4, in combination with a right sided discus protrusion at this level. These findings resulted in bilateral narrowing of the foramina. Radices S1 were compressed along their course due to large bilateral sacral dural ectasia (*Figure 1*).

Neurosurgical intervention was not considered beneficial due to high risk of dural tears. A selective locoregional anaesthetic block of the left L4 nerve root resulted in mild reduction of the radiating pain.

Figure 1 Patient 1: T2 weighted MRI at the age of 64 years.

A: Sagittal MRI images of the lumbosacral spine revealed an kyphosis of segments L3 - L5, with extensive degenerative changes (irregular superior and inferior endplates, osteophytes, bulging of discs), dural ectasia and extensive sacral spinal meningeal cysts. **B:** Transversal MRI images: the right S1 root is compressed by large spinal meningeal cysts and no longer completely surrounded by fat.



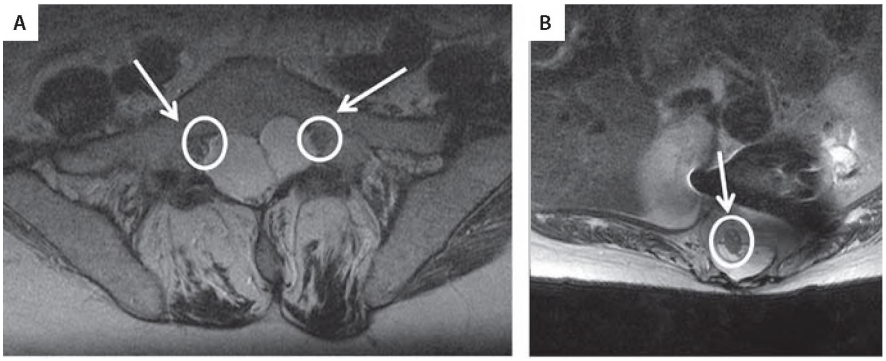
Patient 2

A 58-year female patient suffered from progressive scoliosis, for which a thoracolumbar spondylodesis (anterior fusion and anterior single rod instrumentation Th9 - L2 through a left sided thoraco-abdominal approach) was performed at the age of 33 years. At the age of 49, she reported symptoms of spinal stenosis. She developed a paresis of the right ankle and toe dorsiflexors, for which an orthesis was prescribed. Three years later she experienced numbness of the left foot and ankle. At the age of 56 years weakness of her right foot increased, and the quadriceps and hamstring muscles also became weak. She started using knee braces. One year later, paresis of the gluteus muscles occurred, which further impaired walking. With help of a walking aid she could walk a maximum of 2 km, needing several pauses. Gradually she developed urine retention and constipation for which she started intermittent bladder catheterization and had to remove faeces manually.

Physical examination at the age of 51 years revealed typical signs of Marfan syndrome, including severe kyphoscoliosis of the thoracolumbar spine after surgical correction.

Figure 2 Patient 2: T2 weighted MRI at the age of 58 years: dural ectasia and extensive spinal meningeal cysts at the level of L5 - 1.

A: Transverse MRI images showed a close relationship of the radices S1 and S2 with the dural ectasia and spinal meningeal cysts. Sagittal MRI images further showed a severe thoracolumbar kyphoscoliosis, along which the spinal cord, (epi)conus and cauda were extended. No signs of root compression were found at these levels (not shown). **B:** Transverse MRI images: the posterior surface of the L2 body revealed a large bony defect partially surrounding the conus and seemingly stretching it. Just above that level, susceptibility artefacts due to a ferromagnetic metal implant were observed.



Strength, sensation, and reflexes of her arms, hands, and left leg were normal, but a mild paresis of her right leg MRC grade 4.5 was found. Examination at the age of 58 years showed progressive weakness of her right more than left leg and foot (MRC scores: *Right*: distally 0; hip flexion 3, hip extension 0; hip adduction 4, hip abduction 1, knee extension 3; *Left*: distally 3, proximally 4). Deep tendon reflexes were reduced in the left leg and absent on the right, and vibration sense was reduced in both feet. Muscle atrophy was most pronounced in her right leg, and she had bilateral claw feet (left more than right).

Nerve conduction studies indicated an axonal polyneuropathy. Repeated needle electromyography revealed denervation in several leg muscles (rectus femoris, tibialis anterior, and gastrocnemius bilaterally; right extensor hallucis longus and right gluteus medius), with marked progression of neurogenic changes over the course of seven years. These findings were compatible with a progressive radiculopathy at multiple lumbosacral levels. Sagittal MRI revealed dural ectasia and extensive spinal meningeal cysts at the sacral level. Transverse imaging showed a close relationship of the radices S1 and S2 with the spinal meningeal cysts. Furthermore, sagittal imaging showed severe thoracolumbar kyphoscoliosis, with an elongated aspect of the spinal cord, (epi)conus and cauda at these levels. Furthermore, the body of L2 showed a large bony defect of the posterior surface, partially surrounding the conus. The conus seemed to be stretched out at this level (*Figure 2*).

The patient participated in a clinical rehabilitation program to increase physical condition and to limit impairments. This program consisted of: pelvic floor therapy to improve control of defecation; training with a hand bike to improve outdoor mobility; provision of new orthopaedic shoes; physical therapy including hydrotherapy, focusing on walking and improvement of muscle strength and cardiorespiratory fitness; occupational therapy for adaptive equipment recommendations and usage training, and environmental adaptation including provision of equipment; and psychological assessment and counselling. Based on reduction of her impairments and on the general satisfaction of this patient, this program was successful. Operative intervention including multiple osteotomies of the lumbar spine and instrumentation over a long segment was considered but not performed since its outcome was deemed too unpredictable in relation to the possible complications of the procedure.

Patient 3

This 51-year old male patient suffered from a ruptured aneurysm of the abdominal aorta at the age of 49 years, which resulted in spinal cord ischemia with paraparesis, impotence and urinary retention for which he performed intermittent bladder catheterization. He had gradually regained mobility after rehabilitation, but in spite of the improvement of muscle strength and mobility he experienced progressive pain, radiating from his lower back to both legs and the sacral dermatomes. At rest the pain consisted of a cold burning sensation exacerbated by touch. Walking of short distances (20 m – 100 m) caused a feeling of stiffness in his lower legs that would gradually turn in to severe pain. If he continued walking he would fall because of muscle weakness and pain.

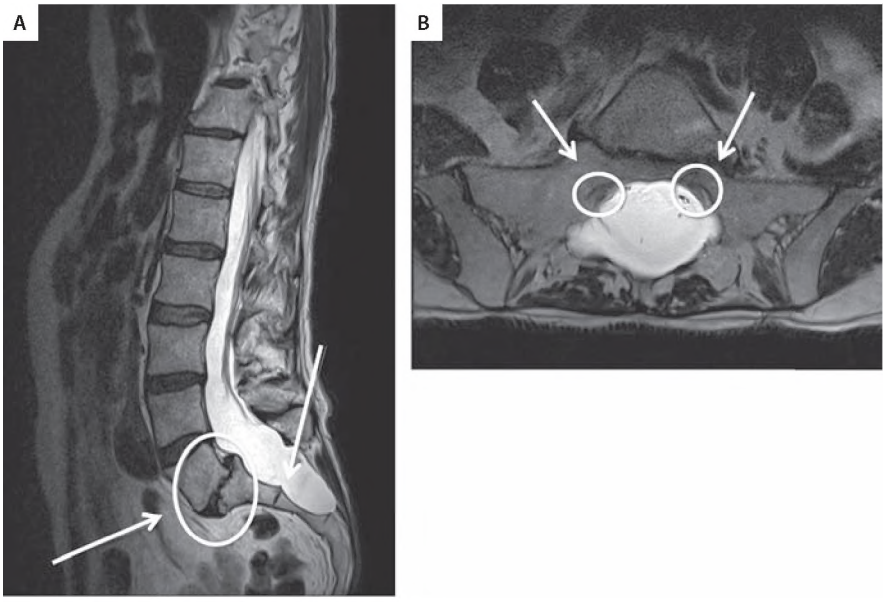
Physical examination showed typical signs of Marfan syndrome. Muscle strength was normal in his arms and reduced in the legs (MRC scores: proximally 4; distally 2-3). Sensation was reduced below the 12th thoracic dermatome. Pain sense and vibration sense were absent in his feet. Touch of the sacral region was very painful locally. Although the knee jerks were increased, his ankle jerks were absent bilaterally.

Nerve conduction studies showed an axonal polyneuropathy. Needle electromyography showed neurogenic changes in the extensor hallucis, tibialis anterior, and gluteus medius muscles on the left, and of the gastrocnemius and gluteus maximus on the right, with signs of reinnervation in the right gluteus maximus. Findings were compatible with an additional longer lasting radiculopathy of the L5 - S2 levels on the left and S1 - S2 levels on the right. No EMG abnormalities were found in the hands. T2 weighted MRI studies of the thoracic, lumbar and sacral spine showed thoracic scoliosis, an anterolisthesis of L5 - S1, and lumbosacral dural ectasia and spinal meningeal cysts with severe scalloping of the posterior surface of S1 and S2, suggestive of longer lasting compression by the dural ectasia. Both S1 roots were in close contact with the dural ectasia and spinal meningeal cysts. No signs of syringomyely were seen (*Figure 3*).

Neurosurgical intervention was again not considered beneficial due to the multifactorial origin of the pain and the high risk of perioperative dural tears.

Figure 3 Patient 3: T2 weighted MRI at the age of 51 years.

A: Sagittal MRI images of the thoracic, lumbar and sacral spine showed an anterolisthesis of L5 - S1, thoracic scoliosis, and lumbosacral dural ectasia and spinal meningeal cysts with severe scalloping of the posterior cortex S1 and S2. **B:** Transverse MRI images: Both S1 roots are in close contact with the dural ectasia and bilateral spinal meningeal cysts.



Discussion

We here report three genetically confirmed older Marfan patients with pain in the lower back and legs and neurological impairment due to progressive radiculopathy at multiple lumbosacral levels. In all patients symptoms occurred predominantly while standing upright or walking, which suggests a role of the hydrostatic pressure of the cerebrospinal fluid within the dural ectasia and/or meningeal cyst in the progression of radicular compression. A concomitant axonal polyneuropathy was detected in all patients, which concurs with our previous findings in ten Marfan patients.[15] The previous spinal cord ischemia has probably contributed to the severity of neurological features in patient 3. Imaging studies revealed (kypho)scoliosis, anterolisthesis, degenerative changes, and sacral root compression by dural ectasia with spinal meningeal cysts in all three patients. Surgical options were considered limited due to the high risk of occurrence of dural tears and osseous complications.

Neurological impairment secondary to spinal abnormalities in Marfan syndrome has occasionally been reported. Hoshino reported a Marfan patient with dysesthesia of dermatome S2 and sacral pain due to S2 root compression by an arachnoid mass.[16] Furthermore, a 25-year old man with a two-year history of worsening distal sensory and motor deficits of the lower limbs due to a large intraspinal arachnoidal cyst was reported by Lazzaro.[19] A study by Foran et al. showed that dural ectasia is often accompanied by leg pain and sensory deficits in the legs, but this was based on questionnaires and did not include clinical or imaging data.[24]

Dural ectasia with or without spinal meningeal cysts may cause symptoms through a variety of mechanisms,[24] including direct pressure on the periosteum and erosion of bony elements of the lumbosacral spine,[25,26] distortion or traction on neural roots,[27] structural thinning and weakening of the sacrum leading to micro-fractures or instability, direct pressure on abdominal organs by anterior meningocele, shifts in cerebrospinal fluid volume, or increased pressure within focal cysts.[28-30] In the three patients reported here, both osseous deformities and dural ectasia with spinal meningeal cysts are likely to have contributed to the radicular dysfunction. An increased vulnerability of the peripheral and central nervous system to mechanical stress by dural ectasia might predispose to these symptoms in Marfan syndrome, similarly as in other inherited connective tissue disorders.[31] Awareness of these complications and development of management protocols is essential since life expectancy of Marfan patients has increased.[7] Marfan syndrome might become better recognized as an inherited connective tissue disorder with potentially severe neurological complications, also at older age.[15]

Reference List

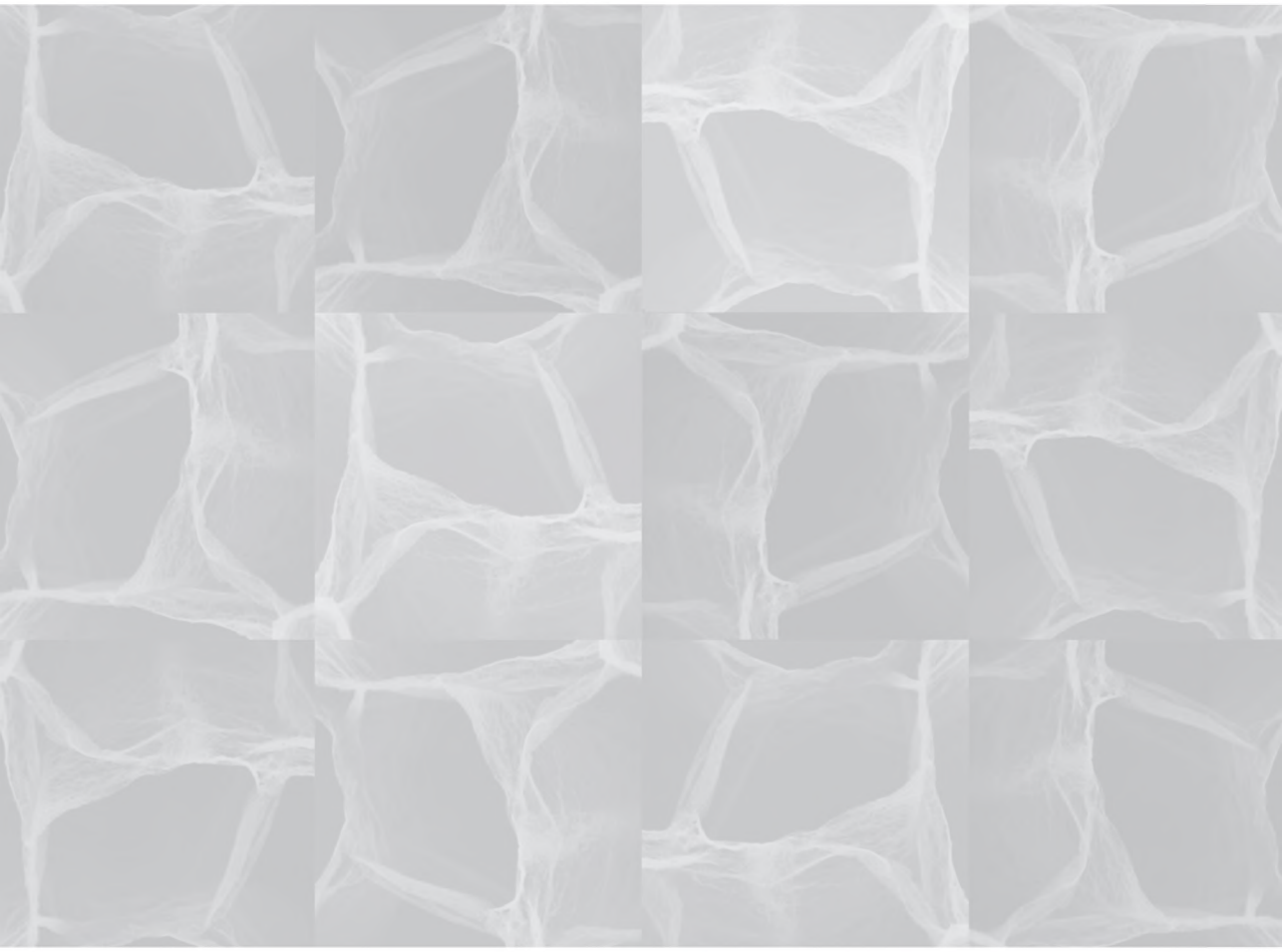
1. Gray JR, Bridges AB, West RR, McLeish L, Stuart AG, Dean JC, Porteous ME, Boxer M, Davies SJ. Life expectancy in British Marfan syndrome populations. Clin Genet 1998; 54: 124-128.
2. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, Boxer M, Devereux RB, Tsipouras P. Life expectancy in the Marfan syndrome. Am J Cardiol 1995; 75: 157-160.
3. von Kodolitsch Y, Robinson PN. Marfan syndrome: an update of genetics, medical and surgical management. Heart 2007; 93: 755-760.
4. Dean JC. Marfan syndrome: clinical diagnosis and management. Eur J Hum Genet 2007; 15: 724-733.
5. Jones KB, Sponseller PD, Erkula G, Sakai L, Ramirez F, Dietz HC, Kost-Byerly S, Bridwell KH, Sandell L. Symposium on the musculoskeletal aspects of Marfan syndrome: meeting report and state of the science. J Orthop Res 2007; 25: 413-422.
6. Ramirez F, Dietz HC. Marfan syndrome: from molecular pathogenesis to clinical treatment. Curr Opin Genet Dev 2007; 17: 252-258.
7. Hasan A, Poloniecki J, Child A. Ageing in Marfan syndrome. Int J Clin Pract 2007; 61: 1308-1320.
8. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
9. Pyeritz RE. Marfan syndrome: 30 years of research equals 30 years of additional life expectancy. Heart 2009; 95: 173-175.
10. De Paepe AM, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996; 62: 417-426.
11. Pyeritz RE, Francke U. The Second International Symposium on the Marfan Syndrome. Am J Med Genet 1993; 47: 127-135.
12. Fattori R, Nienaber CA, Descovich B, Ambrosetto P, Reggiani LB, Pepe G, Kaufmann U, Negrini E, von Kodolitsch Y, Gensini GF. Importance of dural ectasia in phenotypic assessment of Marfan's syndrome. Lancet 1999; 354: 910-913.
13. Villeirs GM, Van Tongerloo AJ, Verstraete KL, Kunnen MF, De Paepe AM. Widening of the spinal canal and dural ectasia in Marfan's syndrome: assessment by CT. Neuroradiology 1999; 41: 850-854.
14. de Kleuver M, van Jonbergen JP, Langeloo DD. Asymptomatic massive dural ectasia associated with neurofibromatosis type 1 threatening spinal column support: treatment by anterior vascularized fibula graft. J Spinal Disord Tech 2004; 17: 539-542.
15. Voermans NC, Timmermans J, van Alfen N, Pillen S, op den Akker J, Lammens M, Zwarts MJ, van Rooij IALM, Hamel BC, Engelen BG. Neuromuscular features in Marfan syndrome. Clin Genet 2009; 76: 25-37.
16. Hoshino Y, Edakuni H, Shimada H, Hayashi S, Machida M, Shimano S, Taya T, Ohki I, Takahashi A, Kurihara T, Yamada I, Arai T, Miyamoto Y, Togo Y. Sacral arachnoid cyst associated with marfan syndrome. Intern Med 2005; 44: 271-273.
17. Nabors MW, Pait TG, Byrd EB, Karim NO, Davis DO, Kobrine AI, Rizzoli HV. Updated assessment and current classification of spinal meningeal cysts. J Neurosurg 1988; 68: 366-377.
18. Oosterhof T, Groenink M, Hulsmans FJ, Mulder BJ, van der Wall EE, Smit R, Hennekam RC. Quantitative assessment of dural ectasia as a marker for Marfan syndrome. Radiology 2001; 220: 514-518.
19. Di Lazzaro V, Pilato F, Dileone M, Minicuci G, Profice P, Colosimo C, Tartaglione T, Tonali PA. Extradural arachnoid cyst with lumbosacral cord and root compression in marfan syndrome. Arch Neurol 2007; 64: 284-285.
20. Sponseller PD, Shindle M. Orthopedic problems in Marfan syndrome. In: Robinson PN, Godfrey M, editors. Marfan Syndrome: a primer for clinicians and scientists. New York: Kluwer Academic / Plenium Publishers; 2004. p. 24-34.
21. Jones KB, Erkula G, Sponseller PD, Dormans JP. Spine deformity correction in Marfan syndrome. Spine 2002; 27: 2003-2012.
22. Robinson L, Dominguez R, Cabrera J, Yeakley JW, Fenstermacher MJ, Milner ME. Multiple meningeal cysts in Marfan syndrome. AJNR Am J Neuroradiol 1989; 10: 1275-1276.
23. Peterson-Kendall F, Kendall-McCreary E, Geise-Provance P, McIntyre-Rodgers M, Romani WA. Muscles testing and Function with Posture and Pain. Baltimore, MD, USA: Lipincott Williams & Wilkins; 2005.
24. Foran JR, Pyeritz RE, Dietz HC, Sponseller PD. Characterization of the symptoms associated with dural ectasia in the Marfan patient. Am J Med Genet A 2005; 134A: 58-65.

25. Stern WE. Dural ectasia and the Marfan syndrome. J Neurosurg 1988; 69: 221-227.
26. Ho NC, Hadley DW, Jain PK, Francomano CA. Case 47: dural ectasia associated with Marfan syndrome. Radiology 2002; 223: 767-771.
27. Smith MD. Large sacral dural defect in Marfan syndrome. A case report. J Bone Joint Surg Am 1993; 75: 1067-1070.
28. Raftopoulos C, Delecluse F, Braude P, Rodesh C, Brotchi J. Anterior sacral meningocele and Marfan syndrome: a review. Acta Chir Belg 1993; 93: 1-7.
29. Sonier CB, Buhe T, Despins P, Delumeau J, de Kersaint-Gilly A. Sacral pseudomeningocele and Marfan's disease. One case. J Neuroradiol 1993; 20: 292-296.
30. Ahn NU, Sponseller PD, Ahn UM, Nallamshetty L, Kuszyk BS, Zinreich SJ. Dural ectasia is associated with back pain in Marfan syndrome. Spine 2000; 25: 1562-1568.
31. Voermans NC, Drost G, van Kampen A, Gabreels-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG. Recurrent neuropathy associated with Ehlers-Danlos syndrome. J Neurol 2006; 253: 670-671.

PART

IV

Summary and outlook



CHAPTER 13

Summary, general discussion, and directions for further research

The last part of this thesis summarizes the results of our clinical and experimental studies; it discusses these findings in the context of other studies and it explores the future research possibilities.

Summary

This thesis is the result of the first systematic study on neuromuscular involvement in two inherited connective tissue disorders (ICTDs), Ehlers-Danlos syndrome (EDS) and Marfan syndrome, and on its underlying pathophysiological mechanism. Neuromuscular symptoms such as muscle weakness, exercise intolerance, easy fatigability, and muscle cramps had only sporadically been reported, and were generally understood to be secondary to reduced physical activity (*Table 1* in *Chapter 4*).[1]

Part I: Introduction and outline

Part I presented a general introduction (*Chapter 1*) and a literature review focussing on the molecular and clinical overlap of ICTDs and myopathies (*Chapter 2*).

Chapter 1: General introduction and outline

Our clinical interest in the neuromuscular features in EDS and Marfan syndrome was raised by the encounter with these patients who were referred for evaluation of a suspected neuromuscular disorder. They suffered from muscle weakness, fatigue or exercise intolerance, and pain. Ancillary investigations excluded a concomitant neuromuscular disorder. Furthermore, the extracellular matrix (ECM) molecules which are deficient in EDS and Marfan syndrome (collagen I, III, and V, tenascin, fibrillin) are distributed in the dense connective tissue network within muscle and peripheral nerve.

The finding that Bethlem myopathy and Ullrich Congenital Muscular Dystrophy (UCMD) are caused by mutations in the gene encoding collagen VI has further increased our attention on neuromuscular features in EDS and Marfan syndrome.[2] Apparently, a primary change in the ECM which surrounds muscle cells significantly influences muscle function. In addition, patients with collagen VI myopathies display hypermobility and skin changes in addition to muscle weakness.[3,4] This points to a clinical overlap of these collagen VI myopathies with the ICTDs.

The encounter with EDS and Marfan patients with neuromuscular symptoms and the discovery of collagen VI myopathies also raised our scientific interest into the physiological mechanisms in which alterations of the muscle ECM influence muscle function. This interest has further grown with current investigations on myofascial force transmission. This concept assumes that the connective tissue within and between the muscles plays a role in transmission of force generated by the myocytes.[5]

The goal of this study was twofold: 1) to explore the occurrence of neuromuscular symptoms in EDS and Marfan syndrome; and 2) to elucidate the role of the ECM in muscle function. Therefore, we defined the following aims (numbering corresponds with the parts of the outline):

(I) to present an overview of the clinical and molecular overlap of ICTDs and myopathies;
(IIA) to study the occurrence and nature of neuromuscular features in EDS;
(IIB) to investigate the pathophysiological mechanisms of muscle weakness in EDS in order to explore the role of the ECM in muscle function, and;
(III) to examine the occurrence of neuromuscular features in Marfan syndrome, to find out whether the findings in part IIA are specific for EDS or can also be found in other ICTDs.
The main findings are summarized.

Chapter 2: Literature review
In *Chapter 2* we presented a literature review on the molecular and clinical overlap of ICTDs and myopathies.[6] Various ICTDs are associated with mild to moderate neuromuscular involvement in addition to the well known dermal, vascular, or articular symptoms. These disorders are caused by defects of matrix-embedded ECM molecules that are also present in the connective tissue within muscle (collagens I, III, V, IX, lysylhydroxylase, tenascin, fibrillin, fibulin, elastin, and perlecan) and interact directly or indirectly with the trans-membrane protein complexes of muscle cells (dystroglycan, integrins, sarcoglycans). Neuromuscular features of ICTDs have so far only sporadically been reported, but can be expected based upon these interactions.

In parallel, a number of myopathies (e.g. congenital muscular dystrophy 1A, 1B or 1C, and Bethlem myopathy) are caused by defects in either sarcolemma-related ECM molecules (α-dystroglycan, sarcoglycan, integrin, and laminin) or matrix-embedded ECM molecules (collagens VI, XIII, and XV). These molecules all interact with other molecules in the ECM network. Clinical characteristics of some of these myopathies imply skin and joint features that are characteristic of ICTDs.

By focusing on the structure, function, and interaction of these ECM molecules, this review pointed to the collective molecular background of these disorders. The clinicians and researchers dealing with (patients with) these myopathies and ICTDs should be aware of this clinical and molecular overlap. Only a multi-disciplinary approach will allow full recognition of the wide variety of symptoms present in this clinical spectrum of ECM defects. Furthermore, joint hypermobility should be recognized as a distinctive feature in the differential diagnosis of myopathies, and as such be of value for all neuromuscular physicians.[7] Hence, this review explored the field of research of the subsequent studies and offered a rationale for them.

Part II: Neuromuscular features of Ehlers-Danlos syndrome
Part IIA: Clinical evaluation of Ehlers-Danlos syndrome patients
The molecular and clinical overlap of ICTDs and myopathies called for a study on the occurrence of neuromuscular features in ICTDs. In part IIA, the results of these case reports and systematic studies on neuromuscular symptoms, fatigue, and pain in EDS were presented.

Chapter 3: Initial clinical observations in Ehlers-Danlos syndrome

In *Chapter 3*, we reported three remarkable EDS cases with various neuromuscular features. The first report describes a 30-year-old female hypermobility type EDS patient with various compression neuropathies: she subsequently suffered from axillary neuropathy, brachial plexopathy, peroneal neuropathy, and sciatic neuropathy.[8] The latter had occurred after having sit cross-legged for several hours. These compression neuropathies were all confirmed by nerve conduction studies and electromyography, and genetic analysis had excluded a hereditary neuropathy with liability to pressure palsies. Peripheral neuropathy in the hypermobility type of EDS may be due to hypermobility of joints which causes an abnormal stretching of or pressure on peripheral nerves, resulting in a neuropathy or plexopathy. In addition, increased vulnerability of peripheral nerves to stretching or pressure due to the genetic ECM defect might be involved. Clinicians and patients should be alert for this possible risk of neuropathies in EDS in order to prevent recurrent nerve injuries with subsequent impairments.[8]

After that, we reported the neuromuscular features of an adolescent patient with the kyphoscoliotic type of EDS due to a homozygous deletion of *PLOD1* (lysine hydroxylase 1;1p36.3-p36.2).[9,10] Muscle hypotonia and weakness had so far only been recognized in neonates with this EDS type, but not later in life. At the age of 16, this patient displayed both proximal and distal muscle weakness and hypotonia. Ancillary investigations revealed signs of myopathy and mild axonal polyneuropathy, both of which may have contributed to muscle weakness. This case report may thus improve recognition of neuromuscular features in the kyphoscoliotic type of EDS beyond the neonatal period, which has to be taken into account into rehabilitation programs for these patients.[10]

The third case is a 50-year-old TNX-deficient type EDS patient, who presented with moderate proximal and severe distal muscle weakness, and generalized joint hypermobility with atrophy and contractures in her hands.[11] Because of muscle weakness, she had been referred to our outpatients department several years before, even prior to EDS was diagnosed. Muscle biopsy had not revealed any myopathic changes initially, and a second biopsy at the age of 50 did neither. The combination of muscle weakness, hypermobility, and contractures also occurs in collagen VI myopathies.[4] Collagen VI is an ECM component that depends on TNX for proper functioning. The muscle biopsy in this patient indeed showed mildly reduced collagen VI staining.[11]

Chapter 4: Systematic clinical observational study on neuromuscular features in Ehlers-Danlos syndrome

The variety of these case reports contributed to the design of the systematic observational study on neuromuscular features in EDS described in *Chapter 4*. Standardized neuromuscular questionnaires, physical examination, nerve conduction studies, electromyography, muscle

ultrasound, and muscle biopsy were performed in 40 EDS patients with the vascular, classic, TNX–deficient, and hypermobility type of EDS caused by *TNXB* haploinsufficiency.[12]

Muscle weakness, myalgia, and easy fatigability were reported by the majority of patients (respectively 65%, 73%, and 60%). Mild-to-moderate muscle weakness and reduction of vibration sense were common (85% and 60% respectively). Nerve conduction studies demonstrated axonal polyneuropathy in five patients (13%) in absence of a metabolic cause of polyneuropathy. Needle electromyography revealed predominantly myopathic features in 23%, and a mixed neurogenic-myopathic pattern in 53% of the patients. Muscle ultrasound showed increased echo-intensity and atrophy in approximately one half of the patients (50% and 48% respectively). Mild myopathic features were seen in muscle biopsies of five patients (28%). Furthermore, qualitative evaluation of electron microscopic images revealed a lower density of collagen fibrils in the endomysium and perimysium in all EDS types; and collagen fibrils were more often short in the biopsies of the classical type EDS. Overall, patients with the hypermobility type EDS caused by *TNXB* haploinsufficiency were least affected, which points to a remarkable relation between residual TNX level and degree of neuromuscular involvement. This dose–effect relation points to a role of the ECM in muscle and peripheral nerve function in these patients.[12]

Chapter 5 and 6: Questionnaire study on fatigue and pain in Ehlers-Danlos syndrome

In order to further assess the prevalence of fatigue and pain in EDS, and the impact of EDS on daily life, we performed a written questionnaire study among 273 members of the Dutch Ehlers-Danlos patient organization (*Chapter 5*)[13,14]

More than three quarter of EDS patients (77%) suffered from severe fatigue.[14] Patients who were severely fatigued were more impaired than non-severely fatigued patients and reported a higher level of psychological distress. The five possible determinants involved in fatigue were sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain severity. These determinants could form a starting point for the development of an effective cognitive behavioural intervention for fatigue in EDS.[14]

Since pain severity was one of the determinants of fatigue, we subsequently analyzed various aspects of pain and their relation with functional impairment and sleep disturbances (*Chapter 6*).[13] The results showed that chronic pain in EDS is highly prevalent (90%) and associated with regular use of analgesics. Moreover, pain was more prevalent and more severe in the hypermobility type than in the classical type, and pain severity in EDS was correlated with hypermobility, dislocations, previous surgery, and with low nocturnal sleep quality. Pain was also found to contribute to functional impairment in daily life, independently of the level of fatigue. Therefore, treatment of pain should be a prominent aspect of symptomatic management of EDS as well.[13]

Part IIB: Quantitative muscle function measurements of tenascin-X-deficient Ehlers-Danlos syndrome patients and tenascin-XB knockout mice

The studies described in Part IIB focused on the pathophysiological mechanisms of muscle weakness in EDS and were performed both TNX-deficient EDS patients and on *Tnxb* knockout (KO) mice. We refer to *Box 3* and *Table 1* in *Chapter 9* for a description of these quantitative muscle force measurements and concepts. *Table 1* in this chapter summarizes and combines the findings of these studies.

Chapter 7: Pilot study on quantitative muscle function in two tenascin-X-deficient Ehlers-Danlos syndrome patients

We started with a pilot study on quantitative muscle function in two TNX-deficient EDS patients and measured knee extension torques at relatively long muscle length (knee flexion 90°) (*Chapter 7*).[16] The results showed reduced maximal torques at voluntary contraction, relatively high twitch torques, a normal delay between stimulation and torque generation, increased rate of torque rise, and normal relaxation.

These findings could not be attributed to reduced physical activity or muscle atrophy alone since physical examination, muscle ultrasound, and muscle biopsy showed no signs of atrophy; and the findings in both patients were very similar, although one of them used a wheelchair.[16] Changes in myotendinous force transmission due to enhanced tendon compliance could not explain the findings either. This latter would have reduced torque generation, have lowered the twitch torques, have increased the delay between stimulation and torque generation, and have delayed relaxation. In contrast, we hypothesized that TNX deficiency reduces the stiffness of (intra- and extramuscular) myofascial pathways, and thus causes a pathological reduction of the force transmitted this way (myofascial force transmission).[16] This would, in its turn, change the required muscle coordination drastically, and interfere with mechanical interaction between antagonistic muscles in physiological movements.[16]

Chapter 8: Standardized neuromuscular assessment of tenascin-XB knockout mice

We subsequently performed a quantitative muscle strength study on *Tnxb* KO mice (*Chapter 9*).[17] This was preceded by a study on the neuromuscular phenotype of these *Tnxb* KO mice in order to better characterize the phenotype of these mice (*Chapter 8*).[18] This study consisted of standardized functional assessment (hangtime and paw-fall-through test, and long-term spontaneous motor activity assessment and quantification), muscle histology, and RNA expression profiling of muscle tissue. In addition, peripheral nerve composition was studied by histology and electronmicroscopy.

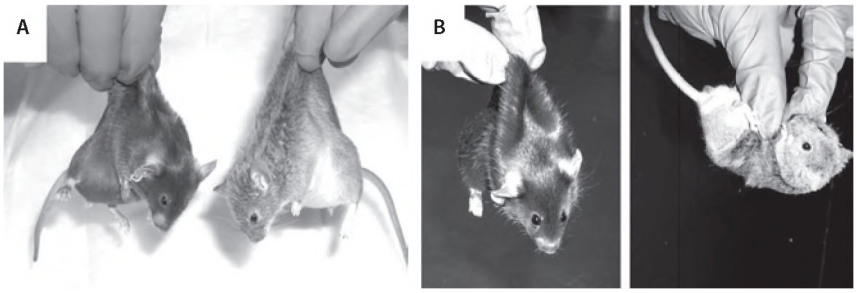
The results showed presence of mild muscle weakness without reduction of long-term spontaneous motor activity, and mild myopathic features in the muscle biopsies. The results

BOX 3 Quantitative muscle assessment.

Quantitative muscle assessment implies various ways to quantitatively measure muscle force. We will shortly describe the concepts and methods used.

Torque	The moment of force; this is the tendency of a force to rotate an object around an axis. The magnitude of torque depends on three quantities: (1) the force applied; (2) the length of the lever arm connecting the axis to the point of force application; and (3) the angle between the two.
Series elastic component	The series elastic component of muscle consists of the ECM within muscle and the tendons attached to the muscle. When the musculotendinous unit is activated the SEC acts as a spring or coil and is lengthened while the muscle fibres shorten. As SEC lengthens, elastic energy is stored. If the active musculotendinous unit shortens after an isometric force generation phase, the stored energy is released, allowing the SEC to contribute to total work production. During relaxation the muscle connective tissue and tendons return to their unstretched configuration.
Compliance	Compliance in muscle physiology is a measure of the tendency of the series elastic component in muscle and tendon to resist recoil toward its original dimensions upon removal of a distending or compressing force. It is the reciprocal of "elastance".
Slack	Looseness or lack of tension; this is a characteristic of the SEC compounds of muscle; it reflects the necessity to stretch the SEC before force can be built up and be transmitted.
Voluntary activation capacity	The capacity of a subject to voluntarily activate one's muscles in comparison with the activation reached after maximal activation (e.g. with electrical stimuation). It reflects the percentage of maximal torque generation capacity that can be generated by voluntarily contraction.
Myofascial force transmission	The ability of muscle to transmit forces between muscle fibres and connective tissue within muscle (endomysium and perimysium) and between individual muscles and connective tissue between muscles (epimysium, fascia, septum, neurovascular tract)

Skin phenotype of *Tnxb* knockout (KO) mice: **A:** six-month-old WT (black) and *Tnxb* KO (grey) littermates were anesthetized and held by the skin of their back. The hyperextensible skin of the *Tnxb* KO mouse is apparent. **B:** when awake mice were again held by the skin of the back: the WT mouse (black) struggled but could not turn; the *Tnxb* KO mouse (grey) turned, climbed his own skin and bit the investigator. Reproduced with permission of NPG.[15]



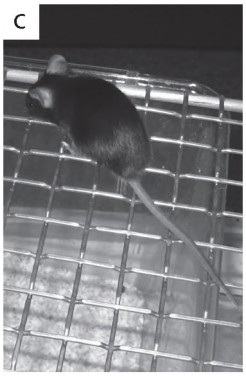
BOX 3 Continued.

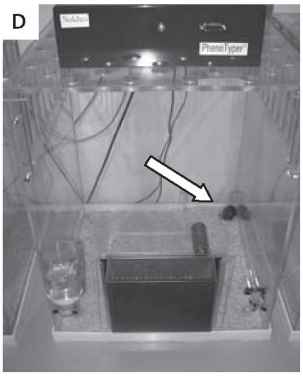
C: Functional strength measurement with use of the paw-fall-through test: during a 1-minute observation period, the number of times individual limbs fell through the wire were counted.

D: Long term motor assessment in a home cage-like environment; the box on top of the plastic cage contains the camera and computer. The mouse is sitting in the left back corner (white arrow). Both experiments are described in *Chapter 8*.

E: Experimental set-up of *series A* of the quantitative muscle function assessment in *Chapter 9*: The distal tendon of the fully dissected medial gastrocnemius muscle is connected to a force transducer (white arrow); the proximal tendon is left intact. Contractions were induced by electrical stimulation of the sciatic nerve (grey arrow). Force measurements were performed by a transducer attached to the distal tendon.

F: Experimental set up of the quantitative measurements in TNX-deficient EDS patients in *Chapter 10*: Knee extension and flexion torques were measured with the subjects seated on a custom-built computer-controlled lower limb dynamometer. The lower leg was connected to the lever arm of the dynamometer (white arrow) with the knee flexed at different angles. Padded straps around the pelvis and upper body minimized undesired movements of the hip.





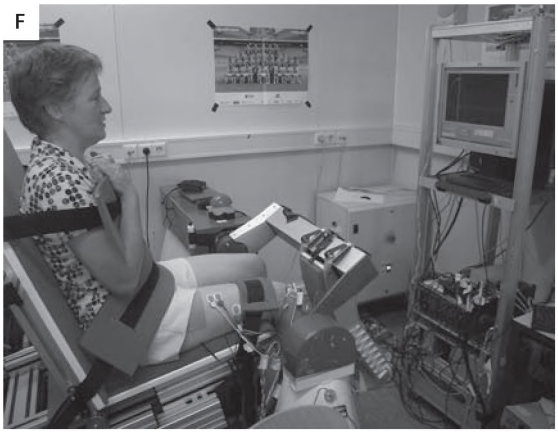


Table 1 Summary and combination of findings of experimental studies in TNX-deficient EDS patients and *Tnxb* KO mice.

Chapter	Most important findings	Interpretation	Hypothesis
Chapter 7 TNX-deficient EDS patients	*Voluntary and stimulated knee extension at 90° knee flexion:* 1) Reduced maximal torques at voluntary contraction; 2) Normal delay between stimulation and contraction, increased torque rise, and normal relaxation rate. At long muscle length.	1) Changes are not due to reduced physical activity, muscle atrophy, or changes in myotendinous force transmission.	1) Changes are most likely caused by changes in myofascial force transmission (Continued in *Chapter 9, series B*).
Chapter 9: *Series A* *Tnxb* KO mice	*Stimulated isometric GM contraction at various lengths:* 3) Lower normalized active isometric force; 4) Longer delay between stimulation and contraction, normal rise of active force, and reduced relaxation rate. Only at short muscle length.	2) Altered series elastic component (i.e. higher compliance) within the maximally dissected GM muscle-tendon complex (both the ECM network of endo- and perimysium and the myotendinous pathway).	Discrepancy between finding 1) at long muscle length and 3) only at short muscle length: 2) Reduced activation capacity may contribute to muscle weakness in EDS (Continued in *Chapter 10*).
Chapter 9: *Series B* *Tnxb* KO mice	*Simultaneously stimulated TA+EHL, EDL and TS contractions at various lengths* 5) TNX deficiency limited the antagonists length-dependent decrease in active force; 6) TNX deficiency significantly affected net (direction and magnitude) epimuscular myofascial force transmission. Only at short muscle length.	3) Reduced myofascial force transmission in TNX deficiency between the muscles in the lower leg, probably due to increased compliance of connective tissue surrounding the individual muscles: muscles in *Tnxb* KO mice act more independently than WT muscles.	3) Alterations in myofascial force transmission probably require adapted patterns of muscular coordination to allow effective physiological movements (To be continued).
Chapter 10: TNX-deficient EDS patients	*Voluntary and stimulated knee extension and flexion at 30°, 60°, and 90° knee flexion:* 1) Reduced maximal voluntary torque of the knee-extensors across all joint angles tested (30° 60° 90°) 2) Normal normalized torques at 30°; 3) Longer delay between stimulation and contraction; 4) Reduced voluntary activation on voluntary contraction, particularly at low muscle length.	Reduced isometric voluntary peak torque in TNX-deficient EDS patients due to: 4) The compliance of the series elastic component of the muscle tissue is increased. 5) Reduced voluntary activation: failure to maximally voluntarily activate the muscles; 6) Muscle length at 30° might not be short enough to detect the changes in the series elastic component.	4) Reduced voluntary activation has been demonstrated in various neuromuscular disorders and in chronic fatigue syndrome. Future research could focus on central and peripheral components of experienced fatigue in EDS (To be continued).

of RNA micro array analysis pointed to a significant upregulation of genes encoding structural ECM components as well as genes involved in synthesis and degradation of the ECM. Additionally, a sciatic nerve sample showed mildly reduced collagen fibril density of the endoneurium, few signs of degeneration and regeneration, and smaller inner and outer diameters of the myelinated fibres in the *Tnxb* KO mouse.[18]

The results of this animal study reinforced the finding of mild to moderate neuromuscular involvement in patients with various types of EDS (*Chapter 4*)[12]. The observations in the sciatic nerve might relate to the occurrence of axonal polyneuropathy in TNX-deficient EDS patients.[12]

Chapter 9: Quantitative muscle strength measurements of tenascin-XB knockout mice

The quantitative muscle strength measurements in *Tnxb* KO mice consisted of two parts.[17,19,20] First, intramuscular aspects of muscle force were studied in isometric contractions of the maximally dissected medial gastrocnemius (GM) muscle (*Series A*). Second, intermuscular aspects of muscle force were explored by testing the force characteristics of the triceps surae muscle (TS) and anterior crural muscles (EDL: extensor digitorum longus; EHL: extensor hallucis longus; and TA: anterior tibial muscles) without major dissection. In this way, mechanical interaction between these muscle groups remained intact and changes in this interaction during contraction could be detected (*Series B*).[17]

The first series of experiments within the maximally dissected GM showed differences only at low muscle lengths (optimum length - 4, - 3.5, - 3 mm): lower normalized active isometric force, a longer delay between stimulus and force rise, and reduced relaxation rate. This pointed to changes in the series elastic component within the maximally dissected GM muscle-tendon complex. This complex includes both the ECM network of endo- and perimysium within the muscle and the myotendinous pathway.[17]

These animal experiments at short muscle length added two important findings to the results of the pilot study. First, the series elastic component is altered at short muscle length, whereas in the pilot study in two TNX-deficient EDS patients with measurements no such observations were made at relatively long muscle length.[16] Changes of the series elastic components would indeed be expected to manifest first at short muscle length, since more slack has to be taken up before the series elastic component can transmit forces. Second, normalized active isometric force in *Tnxb* KO mice was normal at optimum length and above, whereas maximal torque at voluntary contraction in TNX-deficient EDS patients was reduced also at relatively long muscle length. This difference at optimum length between stimulated active isometric force (normal in mice) and maximal torque at voluntary contraction (reduced in patients) raised the question whether reduced activation capacity contributes to muscle weakness in EDS patients.[17]

The second series of experiments in the *Tnxb* KO mice (*Series B*) focussed on the changes of the series elastic component between muscles (consisting of epimysium, neurovascular tracts, and fascia).[17] It showed differences also only at low muscle lengths. In normal mice,

distal active force in the agonistic muscle (TA + EHL and TS respectively) decreased with increasing length of the antagonistic muscle (TS and TA+EHL respectively); this results from myofascial force transmission between these muscle groups.[21] TNX deficiency limited this antagonists length-dependent decrease in active force, which points to reduced myofascial force transmission in TNX deficiency. This is probably due to increased compliance of connective tissue surrounding the individual muscles. Furthermore, TNX deficiency significantly affected net epimuscular myofascial force transmission (measured by difference in force exerted at proximal and distal tendons of the EDL), both in magnitude of the myofascial load and in the direction of loading. Together, these results entail that TNX-deficient muscles are less capable of transmitting forces in other ways than via myotendinous force transmission. In other words, muscles in *Tnxb* KO mice act more independently than wildtype muscles in comparable experimental conditions. Such altered function probably requires adapted patterns of muscular coordination to allow effective physiological movements.[17]

Chapter 10: Quantitative muscle strength measurements in TNX-deficient Ehlers-Danlos syndrome patients

The first step to confirm the findings of *series A* in a non-invasive manner in TNX-deficient patients was to test knee extension torques at short muscle length and to measure voluntary activation capacity. We therefore performed a quantitative muscle function study in seven TNX-deficient EDS patients and five age- and sex-matched control subjects, with assessment of isometric voluntary and electrically elicited contractions (both knee extension and flexion) at 30°, 60° and 90° knee flexion (*Chapter 10*).[22] We controlled for the level of physical activity with a standardized questionnaire in all subjects.

The main findings of this study were that: 1) TNX-deficient EDS patients exhibited reduced maximal voluntary torque of the knee-extensors across all joint angles tested (at 30°, 60°, and 90° knee flexion); 2) normalized torques (normalized to the highest torque at 60°) were not different at 30° and tended to be higher in EDS patients at 90°; 3) the delay between stimulus and torque development was prolonged; and 4) EDS patients exhibited reduced voluntary activation on voluntary contraction, particularly at low muscle length.

Together, these results showed that isometric voluntary peak torque is reduced in TNX-deficient EDS patients, confirming the finding of the pilot study (*Chapter 7*). The increased delay between stimulus and torque development as well as the observation that voluntary activation is radically reduced with low knee-flexion may indicate that the compliance of the series elastic component of the muscle tissue is increased as expected. However, such possible increased compliance seemed not to result in obvious normalized torque reduction at low knee-flexion angle (i.e. short muscle length), although measurements of full torque-angle relations may be required to provide conclusive evidence. Moreover, the

results suggest that an important part of the observed muscle weakness can be explained by failure to maximally voluntarily activate the muscles in addition to these intrinsic changes in the muscle itself.[22] *Table 1* summarizes and combines the findings of the experimental studies.

Part III: Neuromuscular features of Marfan syndrome
Clinical evaluation of Marfan syndrome patients

In order to find out whether the neuromuscular features are specific for EDS or can also be found in other ICTDs[6] we examined the occurrence of neuromuscular features in Marfan syndrome.[23]

Chapter 11: Systematic clinical observational study on neuromuscular features in Marfan syndrome

A systematic observational study on neuromuscular features in Marfan syndrome was performed consisting of a standardized questionnaire, physical examination, nerve conduction studies, needle electromyography, muscle ultrasound, laboratory investigation, and muscle biopsy (*Chapter 11*)[23] In addition, previously performed neuroimages were screened for presence of dural ectasia (i.e. widening or ballooning of the dural sac surrounding the spinal cord, mostly at lumbosacral level) and spinal meningeal cysts.[23]

Various neuromuscular symptoms occur more frequently in Marfan patients than in controls. The four older patients (> 50 years) reported muscle weakness and had functional impairments. Five patients had a mild-to-moderate reduction of vibration sense. The nerve conduction studies showed an axonal polyneuropathy in four patients and needle electromyography revealed myopathic and neurogenic changes in all. Increased echo intensity and muscle atrophy was found in more than one half of the patients on muscle ultrasound. The muscle biopsies obtained in two patients showed myopathic changes in the older, female patient.[23] In addition, signs of lumbosacral radiculopathy were found, which co-occurred with dural ectasia and spinal meningeal cysts.[23] Overall, symptoms were most pronounced in the older patients. These findings should be confirmed in a larger group of patients, but already now indicate a need to further the awareness of neuromuscular involvement and radicular symptoms in this population.

Chapter 12: Subsequent clinical observations of lumbosacral radiculopathy in Marfan syndrome

Since the occurrence of lumbosacral radiculopathy associated with dural ectasia was not encountered in EDS and had only sporadically been reported in literature, this was further investigated in three Marfan patients (*Chapter 12*).[24] Previous studies had shown that dural ectasia occurs in approximately 90% of adult Marfan patients, and that its severity increases with aging.[25] It is probably caused by ongoing hydrostatic pressure and transmitted pulsation of cerebrospinal fluid, which progressively dilates the dural sac.[25] In addition to dural ectasia,

other lumbosacral spinal deformities may contribute to lower back or sacral pain in Marfan syndrome; these include narrow pedicles, thin laminae, vertebral scalloping, and wide transverse processes, as well as kyphoscoliosis and spondylolisthesis.[26,27]

We reported three patients with moderate to severe lumbosacral radiculopathy due to (kypho)scoliosis and dural ectasia with spinal meningeal cysts (*Chapter 12*).[24] The pain, muscle weakness, muscle atrophy, and sensory disturbances illustrate the severe neurological complications which may occur in Marfan syndrome, especially at a later age. Awareness of these complications and the development of management protocols are essential since life expectancy of Marfan patients has increased. Marfan syndrome might gradually become recognized as an ICTD with potentially severe neurological and neuromuscular complications during ageing.[24,28]

General discussion and directions for future research

After the summary of our results, we here discuss the findings in the context of the other earlier and recent studies; and explore future research possibilities.

Part IIA: Clinical evaluation of Ehlers-Danlos syndrome patients
Part III: Clinical evaluation of Marfan syndrome patients

Neuromuscular features in Ehlers-Danlos syndrome and Marfan syndrome (Chapter 3-4 and 11-12)

The occurrence of muscular symptoms in EDS was already observed by Beighton in 1970, who mentioned weak, hypotonic muscles in his comprehensive thesis on EDS.[1] He discussed the findings of previous reports,[29,30] including hypotonia, progressive muscle weakness, poorly developed musculature, and scapular winging, all without signs of concomitant myopathy. Beighton therefore interpreted the findings as secondary to avoidance of exercise because of the hypermobility and instability of joints.[1] Nevertheless, he did discuss the suggestion that the muscle hypotonia might have a basis in an abnormality of the collagen in muscle sheaths rather than in the muscle fibres itself, an idea that was originally reported by Froelich in 1949.[31] This idea was supported by the observation by Melnikov that the connective tissue in the muscles of EDS patients was very sparse, so that the muscle bundles were hardly held together.[1,32] However, since then this concept has gradually received less attention, a development which might be related to the evolution of medical specialities with an exclusively narrowing of the focus of these specialists to the organ or tissue of their specialism.

Similarly, Marfan himself reported muscle hypoplasia in his initial description of the syndrome.[33] It has long been acknowledged as a significant clinical feature and was reported in up to 80% of cases.[34,35] Furthermore, the handbook of McKusick reported in 1966 that "*Muscular underdevelopment and hypotonia is a frequent but by no means invariable feature. This feature has been so striking as to suggest a primary disorder of muscle in some instances.*"[36] However, these features gradually received less attention, and in refining the nosology of Marfan's syndrome in 1979, Pyeritz and McKusick did no longer mention muscular involvement.[37]

Only recently, neuromuscular features in Marfan syndrome have received renewed attention.[38,39] Behan reported a family with Marfan syndrome and mild proximal and distal muscle weakness and respiratory failure.[38] Electromyography showed myopathic units. Muscle biopsy revealed no myopathic changes, but the fibrillin immunostaining was reduced in endo- and perimysium, and the fibrillin present appeared to be a truncated form of the protein. It was suggested that this contributed to the myopathy in this family and that muscle features should be borne in mind when assessing Marfan patients.[38]

Our findings in Marfan patients are in accordance with a recent experimental study on muscle regeneration in a mice model of Marfan syndrome.[39] The results of this study shed light on the pathophysiological mechanism of myopathy in Marfan syndrome: the patho-

physiology of Marfan syndrome involves excessive signalling by transforming growth factor (TGF)-β, which is known to inhibit the terminal differentiation of cultured myoblasts into myocytes. Increased TGF-β activity in Marfan syndrome impairs muscle regeneration in fibrillin-1–deficient mice. This study also showed that systemic antagonism of TGF-β through administration of the angiotensin II type 1 receptor blocker losartan normalizes muscle architecture, repair and function in vivo.[39]

We expect that the findings of this thesis will enhance recognition of the neuromuscular phenotype of both disorders, and possibly also of other ICTDs. Follow-up of EDS and Marfan patients at outpatient's clinics calls for a multi-disciplinary approach that preferably includes neurological examination and attention for neuromuscular and radicular symptoms. Muscle biopsies to exclude the presence of a co-existent myopathy might not be necessary if typical clinical and neurophysiological findings are encountered. *Box 4* and *Figure 1* summarize the clinical features that should prompt the neuro(myo)logist to include EDS in the differential diagnosis, and *Box 5* illustrates how to measure joint hypermobility. Vice versa, neuro(myo) logists could add a standardized assessment of joint mobility with a goniometer to the physical examination of patients suspected of myopathies, since joint hypermobility can be a clue in the differential diagnosis of myopathies.[7]

Neuromuscular symptoms in other inherited connective tissue disorders and myopathies

The clinical and molecular overlap between ICTDs and certain myopathies as well as the presence of neuromuscular symptoms in EDS and Marfan syndrome might suggest that other ICTDs are also associated with neuromuscular symptoms, and subsequently, that this is also related to the underlying ECM defect. This could be investigated systematically in a number of patients with osteogenesis imperfecta, due to collagen I defects or cutis laxa due to elastin defects. Muscle weakness has been reported in both disorders.[6,40-42]

In parallel, experimental studies (neuromuscular assessment and quantitative muscle function measurements) could be extended to include other animal models of ICTDs. Mice models of the vascular type and the kyphoscoliotic type EDS and of Marfan syndrome are available, of which the latter two have been characterized with muscle weakness.[39,43,44] To find out if altered ECM composition in these animals also reduces myofascial force transmission in these mice, more detailed studies are needed.[21] This would further support the concept that the ECM composition influences muscle function.

Another extension of our current study population would be to include patients with collagen VI myopathies. Quantitative muscle function studies in these patients could generate further support for the hypothesis of reduced myofascial force transmission in patients with an altered muscle ECM. This would preferably be tested in patients with Bethlem myopathy, since muscle histology is relatively normal in Bethlem myopathy compared with Ullrich congenital muscular dystrophy.

Peripheral nerve function in Ehlers-Danlos syndrome

The clinical and experimental studies in this thesis focused primarily on muscle symptoms and muscle function in EDS and Marfan syndrome. In addition, various findings suggested that peripheral nerve function is also affected by the ECM defect: the case report of an EDS patient with subsequent compression neuropathies (*Chapter 3*), the finding of axonal polyneuropathy in five EDS patients (*Chapter 4*) and four Marfan patients (*Chapter 11*), and the observation of reduced collagen fibril density of the endoneurium, few signs of degeneration and regeneration, and smaller inner and outer diameters of the myelinated fibres in a *Tnxb* KO mouse (*Chapter 8*). A systematic analysis of peripheral nerve function in a larger number of *Tnxb* KO mice is therefore warranted. Moreover, the study of neuromuscular symptoms in other ICTDs mentioned above should focus on both muscle and peripheral nerve function.

Causes of so far unsolved myopathies and a predominant role of fibroblasts

The search for causes of so far unsolved cases of myopathy might include other ECM molecules. This is especially important if clinical features include dermal, vascular, articular or skeletal features. For example, in a number of patients with a phenotype of UCMD or Bethlem myopathy, no COL6A mutations are found, and other ECM molecules may be involved in their pathophysiology. This also accounts for unexplained cases of ICTDs, such as the hypermobility type EDS. Results of previous interaction studies have suggested ECM molecules that might be involved: e.g. collagen XII,[46] fibronectin,[47] biglycan, and decorin.[6,48]

It has recently been shown that muscle interstitial fibroblasts and not myocytes are the main source of collagen VI synthesis in skeletal muscle.[49] This implies major roles of this cell type and the ECM in the pathogenesis of Bethlem myopathy and Ullrich congenital muscular dystrophy. Fibroblasts may similarly be the providers of other ECM components. This might be investigated further and should be taken into account in future studies on diseases caused by ECM defects.

Fatigue and pain are predominant features of Ehlers-Danlos syndrome

The neuromuscular questionnaires included in the clinical studies (*Chapter 4* and *11*) had revealed that fatigue and (muscle) pain were common among both EDS and Marfan patients.[12,23] However, both symptoms had received only little attention in medical literature for long.[45,50] We therefore analysed these symptoms in an extensive questionnaire study among members of the Dutch patient support group for EDS. This study has added that severe fatigue (77% of EDS patients) and pain (90% of EDS patients) are debilitating features in EDS.[13,14] These results may support EDS patients not only in medical recognition but also in social acceptation of their impairments.[51,52]

The observation that fatigue and pain drastically interfere with daily life is vividly illustrated by a recent patients' account on EDS in the *British Medical Journal*.[51]. These case

Figure 1 Clinical features of EDS.

A, **B**, and **C**:Joint hypermobility and skin hyperextensibility in a patient with the TNX-deficient type EDS. **D:** Wide, atrophic scarring in a patient with the classical type EDS. **E, F** and **H:** Thin translucent skin, joint hypermobility, and typical facial appearance in a patient with the vascular type EDS: (large eyes), small chin, and thin lips. **G:** Atrophic scarring (cigarette paper like scar) in a patient with the classical type EDS.

BOX 4 When to think of EDS.[7,12,45]

General history	Delayed gross motor milestones Double-jointed in sports; being proficient at ballet and gymnastics during childhood Showing hypermobility "tricks" during childhood Giving up sports during the teens due to recurrent injuries, pain, and fatigue Mild generalized muscle weakness Musculoskeletal pain Easy fatigability Paresthesias
Medical history	Recurrent (sub)luxation Tendon and muscle rupture Anal prolaps in childhood Reduced effect of local anaesthesia Complications of surgery, e.g. hernia Easy bruising, sometimes with incorrect suspicion of child abuse Abnormal wound healing with wide atrophic scars Uterus prolaps or cervical insufficiency with premature delivery Early onset varicose veins Pneumothorax or pneumohematothorax Spontaneous arterial rupture (vascular or kyphoscoliotic type) Clubfoot (vascular type) Intestinal or uterine fragility or rupture (vascular type) Severe scoliosis at birth (kyphoscoliotic type) Congenital bilateral hip dislocation (arthrochalasia type)
Family history	Hypermobility Sudden death (vascular type)
Physical examination	Generalized joint hypermobility (Beighton score ≥ 5) Increased skin hyperextensibility (≥ 4 cm on volar side of lower arm) Smooth, velvety skin Wide, atrophic scars Molluscoid pseudotumors (calcifications of superficial hematomas that frequently develop at pressure points like heels, knees, and elbows or are associated with scars) Piezogenic papules (pressure-induced lesions that appear on the heels while bearing weight, due to herniation of fat tissue into the dermis) Sferoids (small subcutaneous hard bodies, frequently mobile and palpable on forearms and shins) Thin, translucent skin, characteristic facial appearance, and acrogeria (vascular type) Gingival recession (vascular type) Microcornea (kyphoscoliotic type) Kyphoscoliosis (kyphoscoliotic or arthrochalasia type)
Neurological examination	Mild proximal weakness Mild reduction of vibration sense (measured with Rydell-Seiffer tuning fork) Reduction but no absence of tendon reflexes
Ancillary investigations	*Nerve conduction studies:* reduction of compound muscle action potential (CMAP) of distal muscles, mild axonal sensomotor polyneuropathy *Electromyography:* mixed pattern of both small (myopathic) and larger (neurogenic) units, or predominantly myopathic units *CK:* normal or mildly elevated (generally < 500 U/l) *Muscle biopsy*: normal or mild myopathic features (increase of fibre diameter variance and internal nuclei) *Imaging (MR, CT, ultrasound)*: mitral valve prolaps (common) or proximal aortic dilatation (uncommon) Bone densitometry: osteopenia (kyphoscoliotic type)

BOX 5 How to measure joint hypermobility.

Beighton score
Degree of mobility by passive manoeuvres in 5 joints.
Total score: 0-9.
Hypermobility:
score ≥ 5.

1) Dorsiflexion of the little fingers beyond 90°; one point for each hand;
2) Apposition of the thumbs to the flexor aspect of the forearm; one point for each hand;
3) Hyperextension of the elbows beyond 10°; one point for each elbow;
4) Hyperextension of the knees beyond 10°; one point for each knee;
5) Forward flexion of the trunk with knees fully extended so that the palms of the hand rest flat on the floor; one point.
One point for each hypermobile joint.

Bulbena score
Degree of mobility by passive manoeuvres in 9 joints.
Total score: 0-10.
Hypermobility:
≥ 5 (women);
≥ 4 (men).

Upper extremity:
1) Thumb: passive apposition of the thumb to the flexor aspect of the forearm < 21 mm;
2) Metacarpophalangeal: with the palm of the hand resting on the table, the passive dorsiflexion of the fifth finger is ≥ 90°;
3) Elbow hyperextension: passive hyperextension of the elbow ≥ 10°;
4) External shoulder rotation; with the upper arm touching the body, and the elbow fixed at 90°; the forearm is taken in external rotation to > 85° of the sagittal plane (shoulder line);
Lower extremity – supine position:
5) Hip abduction: passive hip abduction ≥ 85°;
6) Patellar hypermobility: holding with one hand the proximal end of the tibia, the patella can be moved well to the sides with the other hand;
7) Ankle and feet hypermobility: an excess range of passive dorsiflexion of the ankle eversion of the foot can be produced;
8) Metatarsophalangeal: dorsal flexion of the toe over the diaphysis of the first metatarsal is ≥ 90°;
Lower extremity – prone position:
9) Knee hyperflexion: knee flexion allows the heel to make contact with the buttock.
General:
Presence or absence of ecchymoses (1 point for the presence of ecchymoses).
One point for each hypermobile joint.

Beighton score

Illustration of goniometer used for measurement of angles of joint mobility

histories by a mother of a patient and two patients themselves illustrate the many problems facing patients with EDS in its various forms, among which are frequent (sub)luxations, delayed wound healing, pain, fatigue, delayed diagnosis, and the experience of being ignored by health-care professionals.[51] Moreover, a number of studies on quality of life, pain and fatigue in EDS have been published in parallel with our studies or since then.[53-57] We will shortly discuss this below.

In response to our study on neuromuscular features in EDS, Castori et al. reported a high level of bodily pain, and stressed the importance of differentiating between nociceptive and neuropathic pain in order to develop efficient treatment strategies.[53] In a subsequent study on natural history and manifestations of the hypermobility type EDS, the same author reported high frequency of fatigue (86%).[54] The authors also proposed to delineate three phases in the natural course of the hypermobility type of EDS: a "hypermobility" phase, a "pain" phase, and a "stiffness" phase.[54] This classification has proven very useful in explaining the disease course to patients in our centre. Treatment strategies might be most effective when starting in the first phase, to prevent pain and stiffness.[54] Their group also reported that neuropathic pain is a common feature in EDS.[56] Next, Rombaut et al. investigated 32 female hypermobility type EDS and showed a significant presence of joint pain, joint dislocations, muscle cramps, tendinitis, fatigue and headache, with reduction of level of physical activity and health related quality of life.[55] Furthermore, the memory of not being respected or of not taken seriously is substantial for individuals with EDS and can last for years.[52] This results in lack of trust for the health-care system and difficulties in future encounters with health care.[52]

A multidisciplinary treatment approach of fatigue and pain in Ehlers-Danlos syndrome

The five possible determinants involved in fatigue could form a starting point for the development of an effective cognitive behavioural intervention for fatigue in EDS, preferably within a multidisciplinary approach including cognitive behaviour therapy, physical exercise training program, and symptomatic treatment of pain.[13,14]

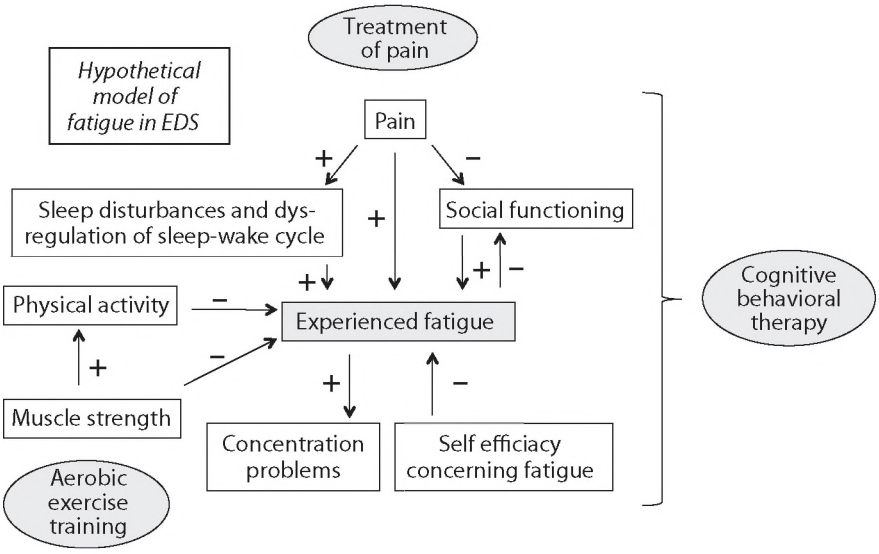
For the design of such an approach, we can use the expertise of our centre on the treatment of fatigue in various neuromuscular disorders. A recent longitudinal study in various neuromuscular disorders has shown that muscle strength, self-reported physical activity, sleep disturbances, and pain at baseline contributed directly or indirectly to fatigue and impairment at follow-up.[58] Lower muscle strength contributed to lower levels of physical activity, which, in turn, contributed to fatigue severity (*Figure 2*). In facioscapulohumeral dystrophy, pain also contributed to physical activity.[58,59] Furthermore, we recently showed that muscle weakness contributes to fatigue also independently of level of activity.[60] Fatigue in EDS may be reduced even more if muscle weakness can also be addressed in the physical exercise training program, in addition to the level of activity.

A multidisciplinary treatment trial of fatigue and pain in EDS could consist of the following three arms (in parallel with a recently designed trial for treatment of chronic fatigue in patients with facioscapulohumeral dystrophy[59]):

1) aerobic exercise training (+ usual care) to improve physical capacity;
2) cognitive behavioural therapy to stimulate an active life-style yet avoiding excessive physical strain (+ usual care); or
3) usual care alone, which consists of no therapy at all or occasional conventional physical therapy.[59] Preferably, adequate symptomatic treatment of pain should also be considered.

Figure 2 A hypothetical model of perpetuating factors of experienced fatigue in EDS, and the multidisciplinary treatment options.

This theoretical model is based on the results of our cross-sectional study on fatigue, in which we identified five possible determinants of fatigue in EDS: sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain. Since we used cross sectional data, we were only able to show that these factors are possible determinants, and a follow-up study would allow analyzing whether these possible determinants are a cause or a consequence of fatigue. Most likely, sleep disturbances, low self-efficacy concerning fatigue, and pain contribute to persistence of severe fatigue. Concentration problems may rather be a result of fatigue, whereas social impairment might be either a perpetuating factor or a consequence of fatigue: increase of pain severity and fatigue negatively influences social participation, whereas strong social support might contribute to persistence of fatigue.



Part IIB: Quantitative muscle function measurements of tenascin-X-deficient EDS patients and tenascin-XB KO mice

Standardized neuromuscular assessment of Tnxb knockout mice

The clinical studies focussed on the occurrence of neuromuscular features in EDS and Marfan syndrome (*Chapter 4* and *11*).[12,23] We continued our research with experimental studies in a animal model of EDS: the *Tnxb* KO mouse.

We choose this mice model of EDS for several reasons. First, it is the model of the TNX-deficient type EDS, the type of which we had recruited and were going to investigate ten patients. In contrast with the classical and hypermobility type EDS, the TNX-deficient type can be confirmed by genetic (*TNXB* mutation or deletion) or biochemical analysis (complete absence of TNX in serum). The diagnosis of the vascular type EDS can also be confirmed by genetic or biochemical testing; however, a mouse model has become available only recently.[44,61] Furthermore, the phenotype of the *Tnxb* KO mice had already been investigated at our centre,[62,63] the mice were still available at our animal laboratory, and we were given the opportunity to investigate their neuromuscular phenotype.

The results of our studies in *Tnxb* KO mice (*Chapter 8*)[18] confirm the observations in the TNX-deficient patients and are in accordance with the findings in a mice model of the kyphoscoliotic type of EDS.[43] These lysyl hydroxylase-1 KO mice were passive and floppy when being handled, with reduction of frequency and strength of spontaneous movements.[43]

Quantitative muscle strength measurements in tenascin-X-deficient Ehlers-Danlos syndrome patients and tenascin-XB knockout mice

The quantitative muscle force measurements in *Tnxb* KO mice point to alterations of the series elastic component (i.e. higher compliance) within the muscle, and to reduced myofascial force transmission (*Chapter 9*).[17] This may drastically affect muscular coordination required for physiological movements. A reduction of the compliance of intra- and extramuscular connective tissue due to the ECM defect results in a contraction in which more slack has to be taken up at the onset of contraction, which will be most prominent at short muscle length. We could not confirm this with the measurements in TNX-deficient EDS patients at 30° (*Chapter 10*), but the muscle might be not short enough in this position to detect this. This study in EDS patients has added that an important part of the observed muscle weakness can probably be explained by failure to maximally voluntarily activate the muscles.

Such a central activation failure has also been demonstrated in various neuromuscular disorders and chronic fatigue syndrome, in which the degree of central activation failure was related to experienced fatigue severity.[64-66] Reduced central activation in EDS and neuromuscular disorders might be a compensatory mechanism in case of abnormal muscle function, i.e. that central activation reduces to prevent overloading of muscles which are

limited in their force generating possibilities. This is a hypothesis that has to be investigated in various neuromuscular disorders and also in EDS.

How can an extracellular matrix defect cause myopathic changes?

The finding of reduced myofascial force transmission, and the suggested altered muscle coordination required for physiological movements probably accounts to some extent for the muscle weakness in EDS. However, we also observed mild myopathic features both in EDS and Marfan patients and in *Tnxb* KO mice. Most likely, an additional pathophysiological mechanism underlying myopathic changes in TNX deficiency is involved. In parallel, how the ECM defect within peripheral nerve alters axonal transport has not been elucidated either.

In *Box 6*, we discuss the concept of cellular tensegrity; it offers a model which might help to understand the effects of extracellular processes on intracellular functioning and vice versa: this concept may support and direct future research questions in this field and may be of assistance in the design of possible investigations.

Changes in intracellular processes in collagen VI myopathies and in Marfan syndrome

In this tensegrity model, defects of ECM molecules logically have direct intracellular effects through a chain of interconnected structural elements. This is illustrated by mitochondrial dysfunction due to defective autophagy in collagen VI myopathies[69] and increased TGF-β activation and signalling in Marfan syndrome.[39,69] Although the collagen VI and the fibrillin deficiency were considered crucial in the pathophysiology of both disorders for a long time, recent research has shown that these ECM defects also have important intracellular effects, which may be the decisive step in the pathophysiological process.[39,69-71] We will discuss these findings below.

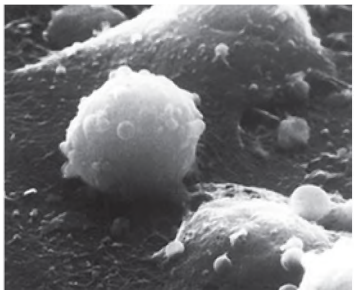
Muscle biopsies of patients with collagen VI myopathies show dysfunctional mitochondria and spontaneous apoptosis, leading to myofibre degeneration.[69] Defects in autophagy of mitochondria were shown to be the cause. Forced activation of autophagy (by genetic, dietary and pharmacological approaches) restored myofibre survival and improved the dystrophic phenotype of COL6A1 KO mice. In addition, in the first part of this discussion we described the increased TGF-β activity in Marfan syndrome which leads to failed muscle regeneration.[39] The effect of losartan on neuromuscular function in Marfan syndrome should be addressed in upcoming clinical trials, preferably both at clinical and at histological level.

Changes in intracellular processes in Ehlers-Danlos syndrome: Titin function

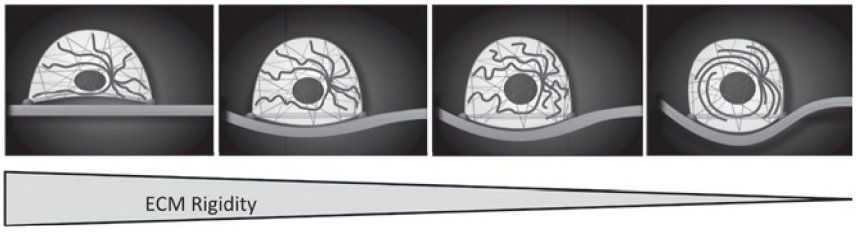
We recently started to investigate intracellular function in EDS patients by studying titin expression and function in muscle biopsies of TNX-deficient EDS patients. Titin (or connectin) is the third myofilament of striated muscle after myosin and actin (*Figure 3* below and *Figure 2* in Chapter 1). It is composed of 244 individually folded protein domains which unfold when

BOX 6 Cellular tensegrity.

Cellular tensegrity is a concept that refers to the balance between tension and structural integrity of living cells. It suggests that a (muscle) cell is a prestressed tensegrity structure; this is that the shape of the (muscle) cell results from the balance of the tension of its various structural components. Tensegrity is a form of tensile architecture that uses tension and compression in a combination that yields strength and resilience beyond the sum of their components. In the cell, stiff hollow fibres within the cytoskeleton called microtubules act as struts, while contractile microfilaments provide tension, acting like stretched rubber bands that compress the microtubules and pull on the ECM through adhesion points. The inward pull of the contractile microfilaments is resisted by microtubules and by the ECM. This matrix is a semi flexible meshwork of large protein filaments that cells anchor to their environment. (http://www.childrenshospital.org/research/cell_tensegrity/index.html).[67,68]



Cellular tensegrity: Cells attach and spread on the ECM by applying traction forces to their adhesion to the matrix.



Making the ECM less rigid (images from left to right side) decreases one of the forces that balances in the microfilaments. The matrix buckles and the whole cell contracts to take on a more round shape. Because the internal microfilaments are unable to bear the forces previously borne by the matrix, they first buckle and then bend around the nucleus as the entire cell membrane retracts and rounds.

the protein is stretched and refold when the tension is removed. Mutations in the gene encoding titin (*TTN* 2q31) are associated with a variety of (cardio)myopathies, among which are dilated cardiomyopathy 1G, hypertrophic cardiomyopathy, tibial distal myopathy, and limb girdle muscular dystrophy 2J.[72]

It is the largest protein known (molecular weight 3,960 kD) and underlies the passive and restorative forces that maintain the structural integrity of the sarcomere and bring about intracellular passive muscle stiffness.[72] As such, it can be considered to be the intracellular counterpart of the elastic components of the ECM which cause the extracellular passive muscle stiffness.

In preliminary studies we found that titin-based stiffness is greatly increased in single muscle fibres from TNX-deficient patients, an increase that might be viewed as an adaptive response to compensate for the reduced ECM stiffness (personal communication with C. Ottenheijm, 2011). In fact, adaptations in titin (becoming more stiff) that counteract opposite changes in the ECM (more loose due to defects of collagen) have been documented previously in dilated cardiomyopathy patients.[73] These exciting findings suggest crosstalk between the ECM and titin, with adaptations in titin that might be beneficial for muscle function. These findings illustrate the tensegrity concept. Vice versa, myopathies with decreased titin-based stiffness may have opposing adaptations of the compliance of the muscle ECM. This might subsequently influence myofascial force transmission. It would be interesting to study this further with quantitative muscle function measurements.

Limitations

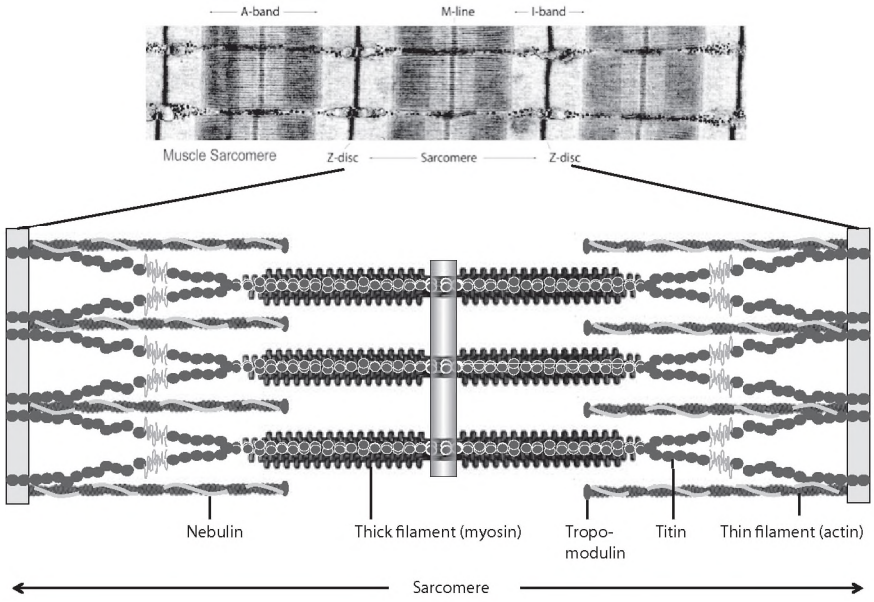
The studies in this thesis have several limitations, which have been discussed in the various chapters and are summarized below.

First, *Chapter 3* contains case-reports of individual patients, and *Chapter 7 and 12* are case series of respectively 2 and 3 patients. A case report is a type of anecdotal evidence. As such, it is less scientifically rigorous than controlled clinical data involving a larger sample size. However, advocates argue that case reports and case series have their own role in the progress of medical science.[74] They permit discovery of new diseases and unexpected effects (adverse or beneficial) as well as the study of mechanisms, and they play an important role in medical education. Case reports and series have a high sensitivity for detecting novelty and therefore remain one of the cornerstones of medical progress; they provide many new ideas in medicine.[74] The case reports on EDS patients (*Chapter 3*) and the case series on three Marfan patients (*Chapter 12*) in this thesis illustrate this role in reporting novel observations, and were the starting point for the subsequent studies. The pilot study in two EDS patients (*Chapter 7*) had a role in the study of the mechanism of muscle weakness in EDS, which was subsequently tested in a mouse model (*Chapter 9*) and in a larger group of TNX-deficient EDS patients (*Chapter 10*). As such, these case reports and case series have contributed the development of the research on this topic.

Another major limitation is that the questionnaire study (*Chapter 5 and 6*) was performed among members of the national EDS patient organisation, and that we only used the questionnaires which were returned. In this way, we were able to include a large number of

Figure 3 Titin is a giant protein that functions as a molecular spring which is responsible for the intracellular passive elasticity of muscle.

As such, titin is important in the contraction of striated muscle tissues. It connects the Z line to the M line in the sarcomere. The protein contributes to force transmission at the Z line and resting tension in the I band region. It limits the range of motion of the sarcomere in tension, thus contributing to the passive stiffness of muscle. Variations in the sequence of titin between different types of muscle (e.g., cardiac or skeletal) has been correlated with differences in the mechanical properties of these muscles.



patients; however, this method includes a selection bias. First, both patients who are not severely impacted by the disease and busy with their work and family, and patients who are severely affected with major impairment might not get involved in a patient support group and tend not to complete questionnaires. Second, we were unable to perform a non-responders analysis, since the patient support group took care of the mailing of the questionnaires and did not send us the names and addresses of their members for privacy reasons. Furthermore, although we only included patients in whom EDS had been diagnosed by a medical specialist, we have not verified these diagnoses ourselves. This included an uncertainty about the diagnosis of the participants of this questionnaire study. However, recruitment of a large number of consecutive EDS patients at an outpatients department would have been complicated, since the care for EDS patients is multidisciplinary, and most hospitals do not have a specialized EDS centre.

Next, the study on mild muscular features in *Tnxb* KO mice (*Chapter 8*) has other limitations. First, we used animals of an advanced age, which raises doubt whether the abnormalities are of pathological or of biological interest. Furthermore, use of animals of different age and different gender for various investigations in the first of these studies may have complicated the interpretation of the results. However, we concluded that due to its explorative design, these results could serve as a starting point for further physiological studies on muscle function in *Tnxb* KO mice. The second experimental animal study (*Chapter 9*) has also used mice of an advanced age, with the same restriction as mentioned above. However, in human, hypermobility generally reduces in aging, and tissues become stiffer; hence, the differences between *Tnxb* KO mice and WT mice might have been larger at a younger age.

Finally, the study on neuromuscular features in Marfan syndrome has been performed in a small group of Marfan patients. This should preferably be confirmed in a larger series. Nevertheless, the findings point to a substantial neuromuscular and radicular involvement in Marfan syndrome. This serves the goal of showing that neuromuscular involvement is not restricted to EDS but also occurs in other ICTDs.

BOX 7 Final conclusions and recommendations.

Final conclusions

1) A clinical and molecular overlap exists between ICTDs and certain myopathies due to defects in molecules of the muscle ECM.
2) Both EDS and Marfan syndrome are associated with various neuromuscular features:
   - Mild neuromuscular features with signs of myopathy and polyneuropathy are part of the phenotype of EDS.
   - Mild neuromuscular features with signs of myopathy, polyneuropathy, and lumbosacral radiculopathy are part of the phenotype of Marfan syndrome.
3) Severe fatigue and chronic pain are highly prevalent among EDS patients (77% and 90% respectively).
   - The five possible determinants involved in fatigue in EDS are sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain severity.
   - Pain is related to hypermobility, dislocations, and previous surgery and associated with moderate to severe impairment in daily functioning.
4) Muscle weakness in EDS is not caused by reduced physical activity but results from:
   - Alterations of the series elastic component of myotendinous pathways (i.e. a higher compliance).
   - A reduction of myofascial force transmission between muscles, due to which muscles act more independently (in *Tnxb* KO mice).
   - A failure to maximally voluntarily activate the muscles (in TNX-deficient patients).

Recommendations for clinical practice

1) Clinical geneticists should pay attention to neuromuscular and radicular symptoms in ICTDs. Vice versa, neuromyologists should look for 'connective tissue symptoms' (joint hypermobility, abnormal scar formation, vascular fragility) in patients with myopathies caused by mutations in genes encoding ECM molecules.[7] They should preferably use a goniometer to measure joint hypermobility.
2) If mild neuromuscular features are encountered in EDS or Marfan syndrome, similarly as what we have described and without other signs of a concomitant myopathy, a muscle biopsy does not have to performed.
3) The five possible determinants involved in fatigue could form a starting point for the development of an effective cognitive behavioural intervention for fatigue in EDS. Treatment of pain should be a prominent aspect of symptomatic management of EDS.

Recommendations for future research

1) Investigate the occurrence of neuromuscular symptoms in other ICTDs.
2) Investigate quantitative muscle function in collagen VI myopathies, preferably in patients with Bethlem myopathy.
4) Consider ECM molecules as causative agents of so far unsolved myopathies.
3) Study the occurrence and pathophysiological mechanisms of peripheral nerve function in EDS and Marfan syndrome in more detail.
5) Investigate how the ECM defects in various types of EDS cause intracellular - both myopathic and axonal - changes. This includes further study of the function of titin in EDS.
6) Explore why central activation capacity is reduced in EDS.

References

1. Beighton P. The Ehlers-Danlos syndromes. London: William Heineman Medical Books Limited; 1970.
2. Jobsis GJ, Keizers H, Vreijling JP, de Visser M, Speer MC, Wolterman RA, Baas F, Bolhuis PA. Type VI collagen mutations in Bethlem myopathy, an autosomal dominant myopathy with contractures. Nat Genet 1996; 14: 113-115.
3. Lampe AK, Bushby KM. Collagen VI related muscle disorders. J Med Genet 2005; 42: 673-685.
4. Kirschner J, Hausser I, Zou Y, Schreiber G, Christen HJ, Brown SC, Anton-Lamprecht I, Muntoni F, Hanefeld F, Bonnemann CG. Ullrich congenital muscular dystrophy: connective tissue abnormalities in the skin support overlap with Ehlers-Danlos syndromes. Am J Med Genet A 2005; 132: 296-301.
5. Huijing PA, Baan GC. Myofascial force transmission via extramuscular pathways occurs between antagonistic muscles. Cells Tissues Organs 2008; 188: 400-414.
6. Voermans NC, Bonnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord 2008; 18: 843-856.
7. Voermans NC, Bonnemann CG, Hamel BC, Jungbluth H, van Engelen BG. Joint hypermobility as a distinctive feature in the differential diagnosis of myopathies. J Neurol 2009; 256: 13-27.
8. Voermans NC, Drost G, van Kampen A, Gabreels-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG. Recurrent neuropathy associated with Ehlers-Danlos syndrome. J Neurol 2006; 253: 670-671.
9. Voermans NC, van Engelen BG. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord 2008; 18: 906.
10. Voermans NC, Bonnemann CG, Lammens M, van Engelen BG, Hamel BC. Myopathy and polyneuropathy in an adolescent with the kyphoscoliotic type of Ehlers-Danlos syndrome. Am J Med Genet A 2009; 149A: 2311-2316.
11. Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Ehlers-Danlos syndrome due to tenascin-X deficiency: Muscle weakness and contractures support overlap with collagen VI myopathies. Am J Med Genet A 2007; 143: 2215-2219.
12. Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol 2009; 65: 687-697.
13. Voermans NC, Knoop H, Bleijenberg G, van Engelen BG. Pain in Ehlers-Danlos syndrome is common, severe, and associated with functional impairment. J Pain Symptom Manage 2010; 40: 370-378.
14. Voermans NC, Knoop H, van de Kamp N, Hamel BC, Bleijenberg G, van Engelen BG. Fatigue is a frequent and clinically relevant problem in Ehlers-Danlos Syndrome. Semin Arthritis Rheum 2010; 40: 267-274.
15. Mao JR, Taylor G, Dean WB, Wagner DR, Afzal V, Lotz JC, Rubin EM, Bristow J. Tenascin-X deficiency mimics Ehlers-Danlos syndrome in mice through alteration of collagen deposition. Nat Genet 2002; 30: 421-425.
16. Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord 2007; 17: 597-602.
17. Huijing PA, Voermans NC, Baan GC, Buse TE, van Engelen BG, de Haan A. Muscle characteristics and altered myofascial force transmission in tenascin-X-deficient mice, a mouse model of Ehlers-Danlos syndrome. J Appl Physiol 2010; 109: 986-995.
18. Voermans NC, Verrijp K, Eshuis L, Balemans MMC, Egging D, Sterrenburg E, van Rooy IALM, van der Laak JWAM, Schalkwijk J, van der Maarel SM, Lammens M, Engelen BG. Mild muscular features in tenascin-X knockout mice, a model of Ehlers-Danlos syndrome. Connect Tissue Res 2011; Mar 15 [Epub ahead of print].
19. Huijing PA. Epimuscular myofascial force transmission between antagonistic and synergistic muscles can explain movement limitation in spastic paresis. J Electromyogr Kinesiol 2007; 17: 708-724.
20. Rijkelijkhuizen JM, Baan GC, de Haan A, de Ruiter CJ, Huijing PA. Extramuscular myofascial force transmission for in situ rat medial gastrocnemius and plantaris muscles in progressive stages of dissection. J Exp Biol 2005; 208: 129-140.
21. Huijing PA. Epimuscular myofascial force transmission: a historical review and implications for new research. International Society of Biomechanics Muybridge Award Lecture, Taipei, 2007. J Biomech 2009; 42: 9-21.
22. Gerrits K, Voermans NC, de Haan A, Engelen BG. Influence of Tenascin-X deficiency on neuromuscular properties of the thigh muscles: a quantitative study in Ehlers-Danlos syndrome. Submitted 2011.

23. Voermans NC, Timmermans J, van Alfen N, Pillen S, op den Akker J, Lammens M, Zwarts MJ, van Rooij I, Hamel BC, van Engelen BG. Neuromuscular features in Marfan syndrome. Clin Genet 2009; 76: 25-37.
24. Voermans NC, Hosman AJ, van Alfen N, Bartels RH, de Kleuver M, op den Akker JW, van Engelen BG. Radicular dysfunction due to spinal deformities in Marfan syndrome at older age: three case reports. Eur J Med Genet 2010; 53: 35-39.
25. Fattori R, Nienaber CA, Descovich B, Ambrosetto P, Reggiani LB, Pepe G, Kaufmann U, Negrini E, von Kodolitsch Y, Gensini GF. Importance of dural ectasia in phenotypic assessment of Marfan's syndrome. Lancet 1999; 354: 910-913.
26. Jones KB, Erkula G, Sponseller PD, Dormans JP. Spine deformity correction in Marfan syndrome. Spine 2002; 27: 2003-2012.
27. Jones KB, Sponseller PD, Erkula G, Sakai L, Ramirez F, Dietz HC, Kost-Byerly S, Bridwell KH, Sandell L. Symposium on the musculoskeletal aspects of Marfan syndrome: meeting report and state of the science. J Orthop Res 2007; 25: 413-422.
28. Voermans NC. Dural ectasia in Marfan syndrome. Neurology 2009; 73: 2047-2048.
29. Brown A, Stock VF. Dermatorrhexis: report of a case. Am J Dis Child 1937; 956.
30. Pittinos GE. Ehlers-Danlos syndrome with a disturbance of creatinine metabolism. J Pediat 1941; 19: 85.
31. Froelich H. Fibrodysplasia elastica generalisata (cutis laxa) and Nervensystem. Nervenartz 1949; 20: 366.
32. Melnikov S, Gorbacheva FE. Clinical features of the Elhers-Danlos syndrome. Vestn derm vener 1965; 1: 83.
33. Marfan AB. Un cas de déformation congénitale des quatres membres, plus prononcée aux extrémités caractérisée par l'allongement des os avec un certain degrée d'amincissement. Bull Mem Soc Med Hop (Paris) 1896; 13: 220-226.
34. Fulcher PH, Southworth H. Arachnodactyly and its medical complications. Arch Intern Med 1938; 61: 693-703.
35. Lambie CG, Shellshear KE, Shellshear JL. Arachnodactyly or Marfan's syndrome. Med J Austr 1950; 1: 213-223.
36. McKusick VA. The Marfan syndrome. Heritable disorders of connective tissues. St.Louis: Mosby Kimpton; 1966. p. 52.
37. Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med 1979; 300: 772-777.
38. Behan WM, Longman C, Petty RK, Comeglio P, Child AH, Boxer M, Foskett P, Harriman DG. Muscle fibrillin deficiency in Marfan's syndrome myopathy. J Neurol Neurosurg Psychiatry 2003; 74: 633-638.
39. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin II type 1 receptor blockade attenuates TGF-beta-induced failure of muscle regeneration in multiple myopathic states. Nat Med 2007; 13: 204-210.
40. Engelbert RH, Beemer FA, van der Graaf Y, Helders PJ. Osteogenesis imperfecta in childhood: impairment and disability--a follow-up study. Arch Phys Med Rehabil 1999; 80: 896-903.
41. Engelbert RH, Uiterwaal CS, Gerver WJ, van der Net JJ, Pruijs HE, Helders PJ. Osteogenesis imperfecta in childhood: impairment and disability. A prospective study with 4-year follow-up. Arch Phys Med Rehabil 2004; 85: 772-778.
42. Morava E, Wopereis S, Coucke P, Gillessen-Kaesbach G, Voit T, Smeitink J, Wevers R, Grunewald S. Defective protein glycosylation in patients with cutis laxa syndrome. Eur J Hum Genet 2005; 13: 414-421.
43. Takaluoma K, Hyry M, Lantto J, Sormunen R, Bank RA, Kivirikko KI, Myllyharju J, Soininen R. Tissue-specific changes in the hydroxylysine content and cross-links of collagens and alterations in fibril morphology in lysyl hydroxylase 1 knock-out mice. J Biol Chem 2007; 282: 6588-6596.
44. Cooper TK, Zhong Q, Krawczyk M, Tae HJ, Muller GA, Schubert R, Myers LA, Dietz HC, Talan MI, Briest W. The haploinsufficient col3a1 mouse as a model for vascular ehlers-danlos syndrome. Vet Pathol 2010; 47: 1028-1039.
45. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet 1998; 77: 31-37.
46. Veit G, Hansen U, Keene DR, Bruckner P, Chiquet-Ehrismann R, Chiquet M, Koch M. Collagen XII interacts with avian tenascin-X through its NC3 domain. J Biol Chem 2006; 281: 27461-27470.
47. Egging D, van den Berkmortel F, Taylor G, Bristow J, Schalkwijk J. Interactions of human tenascin-X domains with dermal extracellular matrix molecules. Arch Dermatol Res 2007; 298: 389-396.

48. Zanotti S, Negri T, Cappelletti C, Bernasconi P, Canioni E, Di Blasi C, Pegoraro E, Angelini C, Ciscato P, Prelle A, Mantegazza R, Morandi L, Mora M. Decorin and biglycan expression is differentially altered in several muscular dystrophies. Brain 2005; 128: 2546-2555.
49. Zou Y, Zhang RZ, Sabatelli P, Chu ML, Bonnemann CG. Muscle interstitial fibroblasts are the main source of collagen VI synthesis in skeletal muscle: implications for congenital muscular dystrophy types Ullrich and Bethlem. J Neuropathol Exp Neurol 2008; 67: 144-154.
50. Sacheti A, Szemere J, Bernstein B, Tafas T, Schechter N, Tsipouras P. Chronic pain is a manifestation of the Ehlers-Danlos syndrome. J Pain Symptom Manage 1997; 14: 88-93.
51. Gawthrop F, Mould R, Sperritt A, Neale F. Ehlers-Danlos syndrome. BMJ 2007; 335: 448-450.
52. Berglund B, Anne-Cathrine M, Randers I. Dignity not fully upheld when seeking health care: experiences expressed by individuals suffering from Ehlers-Danlos syndrome. Disabil Rehabil 2010; 32: 1-7.
53. Castori M, Camerota F, Celletti C, Grammatico P, Padua L. Quality of life in the classic and hypermobility types of Ehlers-Danlos syndrome. Ann Neurol 2010; 67: 145-146.
54. Castori M, Camerota F, Celletti C, Danese C, Santilli V, Saraceni VM, Grammatico P. Natural history and manifestations of the hypermobility type Ehlers-Danlos syndrome: a pilot study on 21 patients. Am J Med Genet A 2010; 152A: 556-564.
55. Rombaut L, Malfait F, Cools A, De PA, Calders P. Musculoskeletal complaints, physical activity and health-related quality of life among patients with the Ehlers-Danlos syndrome hypermobility type. Disabil Rehabil 2010; 32: 1339-1345.
56. Camerota F, Celletti C, Castori M, Grammatico P, Padua L. Neuropathic Pain is a Common Feature in Ehlers-Danlos Syndrome. J Pain Symptom Manage 2010; 67: 145-146.
57. Maeland S, Assmus J, Berglund B. Subjective health complaints in individuals with Ehlers-Danlos syndrome: A questionnaire study. Int J Nurs Stud 2010 Epub ahead of print.
58. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. The development of a model of fatigue in neuromuscular disorders: a longitudinal study. J Psychosom Res 2007; 62: 571-579.
59. Voet NB, Bleijenberg G, Padberg GW, van Engelen BG, Geurts AC. Effect of aerobic exercise training and cognitive behavioural therapy on reduction of chronic fatigue in patients with facioscapulohumeral dystrophy: protocol of the FACTS-2-FSHD trial. BMC Neurol 2010; 10: 56.
60. Voermans NC, Knoop H, Bleijenberg G, Engelen BG. Fatigue is associated with muscle weakness in Ehlers-Danlos syndrome: an explorative study. Physiotherapy 2011; 97: 170-174.
61. Smith LB, Hadoke PW, Dyer E, Denvir MA, Brownstein D, Miller E, Nelson N, Wells S, Cheeseman M, Greenfield A. Haploinsufficiency of the murine Col3a1 locus causes aortic dissection: a novel model of the vascular type of Ehlers-Danlos syndrome. Cardiovasc Res. 2011; 90: 182-90.
62. Egging D, van Vlijmen-Willems I, van Tongeren T., Schalkwijk J, Peeters A. Wound healing in tenascin-X deficient mice suggests that tenascin-X is involved in matrix maturation rather than matrix deposition. Connect Tissue Res 2007; 48: 93-98.
63. Egging DF, van Vlijmen-Willems I, Choi J, Peeters AC, van Rens D, Veit G, Koch M, Davis EC, Schalkwijk J. Analysis of obstetric complications and uterine connective tissue in tenascin-X-deficient humans and mice. Cell Tissue Res 2008; 332: 523-532.
64. Schillings ML, Kalkman JS, Janssen HM, van Engelen BG, Bleijenberg G, Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. Clin Neurophysiol 2007; 118: 292-300.
65. Schillings ML, Stegeman DF, Zwarts MJ. Determining central activation failure and peripheral fatigue in the course of sustained maximal voluntary contractions: a model-based approach. J Appl Physiol 2005; 98: 2292-2297.
66. Schillings ML, Kalkman JS, van der Werf SP, van Engelen BG, Bleijenberg G, Zwarts MJ. Diminished central activation during maximal voluntary contraction in chronic fatigue syndrome. Clin Neurophysiol 2004; 115: 2518-2524.
67. Ingber DE. Tensegrity I. Cell structure and hierarchical systems biology. J Cell Sci 2003; 116: 1157-1173.

68. Ingber DE. Tensegrity II. How structural networks influence cellular information processing networks. J Cell Sci 2003; 116: 1397-1408.
69. Angelin A, Tiepolo T, Sabatelli P, Grumati P, Bergamin N, Golfieri C, Mattioli E, Gualandi F, Ferlini A, Merlini L, Maraldi NM, Bonaldo P, Bernardi P. Mitochondrial dysfunction in the pathogenesis of Ullrich congenital muscular dystrophy and prospective therapy with cyclosporins. Proc Natl Acad Sci U S A 2007; 104: 991-996.
70. Grumati P, Coletto L, Sabatelli P, Cescon M, Angelin A, Bertaggia E, Blaauw B, Urciuolo A, Tiepolo T, Merlini L, Maraldi NM, Bernardi P, Sandri M, Bonaldo P. Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. Nat Med 2010; 16: 1313-1320.
71. Merlini L, Angelin A, Tiepolo T, Braghetta P, Sabatelli P, Zamparelli A, Ferlini A, Maraldi NM, Bonaldo P, Bernardi P. Cyclosporin A corrects mitochondrial dysfunction and muscle apoptosis in patients with collagen VI myopathies. Proc Natl Acad Sci U S A 2008; 105: 5225-5229.
72. Ottenheijm CA, Granzier H. Role of titin in skeletal muscle function and disease. Adv Exp Med Biol 2010; 682: 105-122.
73. LeWinter MM, Granzier H. Cardiac titin: a multifunctional giant. Circulation 2010; 121: 2137-2145.
74. Vandenbroucke JP. In defense of case reports and case series. Ann Intern Med 2001; 134: 330-334.

CHAPTER 14

Nederlandse samenvatting

Het laatste deel van dit proefschrift bevat een samenvatting van de belangrijkste bevindingen.

Samenvatting

Dit proefschrift is het resultaat van het eerste systematische onderzoek naar neuromusculaire symptomen in een ongeselecteerde groep patiënten met Ehlers-Danlos syndroom (EDS) en Marfan syndroom. Dit zijn de twee meest voorkomende erfelijke bindweefselaandoeningen. Het onderzoek richt zich zowel op de aard en frequentie als op het pathofysiologisch mechanisme van deze symptomen. Een aantal neuromusculaire symptomen zoals spierzwakte, inspanningsintolerantie, snelle vermoeidheid en spierkrampen is eerder beschreven in individuele patiënten of families met EDS of Marfan syndroom. Tot nu toe werd dit geweten aan de verminderde fysieke activiteit van patiënten ten gevolge van pijn en angst voor dislocaties (zie ook *Tabel 1* in *Hoofdstuk 4)*.

Deel I: Inleiding en opzet van dit onderzoek

In het eerste deel van dit proefschrift wordt toegelicht wat bindweefsel en extracellulaire matrix (ECM) is en waar dit gelokaliseerd is in de spier. De belangrijkste kenmerken van EDS en Marfan syndroom worden besproken, en er wordt uitgelegd hoe dit onderzoek tot stand is gekomen en is opgezet (*Hoofdstuk 1)*. Verder wordt er een overzicht gegeven van de moleculen waaruit het bindweefsel in spier is opgebouwd, en welke ziekten veroorzaakt kunnen worden door afwijkingen hiervan.

Hoofdstuk 1: Algemene inleiding

Onze interesse in de neuromusculaire symptomen van patiënten met EDS en Marfan syndroom komt voort uit het contact met een aantal patiënten dat naar onze polikliniek werd verwezen met een verdenking op een spier- of zenuwziekte. Zij hadden last van spierpijn, vermoeidheid of pijn. Aanvullend onderzoek liet geen aanwijzingen zien voor een primaire spier- of zenuwziekte, maar wel voor EDS of Marfan syndroom. Inmiddels is bekend dat de moleculen die veranderd of verminderd aanwezig zijn bij patiënten met EDS en Marfan syndroom ook aanwezig zijn in het bindweefsel in de spieren. Dit zou de functie van de spieren kunnen beïnvloeden.

Een aantal jaren geleden werd ontdekt dat bepaalde spierziekten (Bethlem myopathie en Ullrich congenitale spierdystrofie (UCMD)) veroorzaakt worden door een defect in collageen VI, een molecuul in het bindweefsel van spier. Blijkbaar kan een primair bindweefseldefect het functioneren van die spier beïnvloeden. Geleidelijk aan is er ook meer aandacht gekomen voor de huid- en gewrichtskenmerken (toegenomen beweeglijkheid van de distale gewrichten en een abnormale littekenvorming) van deze collageen VI myopathieën. Het voorkomen van deze symptomen bij patiënten met een spierziekte vestigt de aandacht op het klinische en moleculaire continuüm van erfelijke bindweefselaandoeningen en bepaalde spierziekten. Verder heeft recent onderzoek in proefdieren en

patiënten laten zien dat het bindweefsel in en tussen de spieren een rol speelt in de overdracht van kracht die gegenereerd wordt door de individuele spiercellen. Dit wordt myofasciale krachtstransmissie genoemd. Hierdoor is onze interesse in de neuromusculaire symptomen van patiënten met een erfelijke bindweefselziekte nog verder toegenomen.

Door de bovengenoemde interesses heeft dit onderzoek twee doelen; 1) om te onderzoeken hoe vaak en welke neuromusculaire symptomen voorkomen in EDS en Marfan syndroom; en 2) om na te gaan hoe spierzwakte in EDS ontstaat en zo ook duidelijker te maken welke rol bindweefsel speelt in het functioneren van spieren. Dit heeft geleidt tot de volgende subdoelen (de nummering komt overeen met de delen van dit proefschrift):

(I) een overzicht te geven van het klinische en moleculaire continuüm van erfelijke bindweefselziekten en spierziekten veroorzaakt door bindweefseldefecten;

(IIA) te onderzoeken welke neuromusculaire klachten voorkomen bij EDS patiënten, en hoe vaak;

(IIB) te onderzoeken welke pathofysiologische mechanismen een rol spelen in het ontstaan van spierzwakte in EDS om beter te begrijpen waarom EDS gepaard kan gaan met spierzwakte en om meer te leren over de rol van de extracellular matrix in spier;

(III) te onderzoeken welke neuromusculaire klachten voorkomen bij Marfan syndroom patiënten, en hoe vaak om na te gaan of de bevindingen specifiek zijn voor EDS of gegeneraliseerd kunnen worden naar andere erfelijke bindweefselziekten.

De belangrijkste bevindingen worden in dit hoofdstuk samengevat.

Hoofdstuk 2: Overzicht van de literatuur

In hoofdstuk 2 wordt een overzicht gepresenteerd van de literatuur over het moleculaire en klinische continuüm van erfelijke bindweefselziekten en spierziekten veroorzaakt door een defect in de ECM in spier. Er zijn verschillende erfelijke bindweefselziekten die gepaard gaan met lichte tot matige spier- en zenuwbetrokkenheid, naast de bekende huid- en gewrichts- en vasculaire problemen. Deze aandoeningen worden veroorzaakt door defecten van ECM moleculen die ook aanwezig zijn in het bindweefsel in spieren (collageen I, III, V, IX, lysylhydroxylase, tenascine-X (TNX), fibrillin, fibuline, elastine, en perlecan), of die direct of indirect contact maken met de eiwitcomplexen op de oppervlakte van spiercellen (dystroglycan, integrine, sarcoglycan). Hiervan uitgaande zou verwacht kunnen worden dat neuromusculaire symptomen van deze erfelijke bindweefselziekten vaker gerapporteerd worden dan feitelijk het geval is.

Daarnaast wordt een aantal myopathieën (o.a. congenital muscular dystrophy 1A, 1B of 1C, en Bethlem myopathy / UCMD) veroorzaakt door defecten van moleculen die contact maken met de membraan van spiercellen (α-dystroglycan, sarcoglycan, integrine, en laminin), of door defecten van moleculen die meer in de matrix verweven zijn (collageen VI, XIII, en XV). Deze moleculen vormen een netwerk met uitgebreide onderlinge interacties. Beide

groepen van aandoeningen gaan gepaard met een enerzijds 'bindweefselsymptomen' (hypermobiliteit, contracturen, huidafwijkingen, algehele kwetsbaarheid van weefsels) en anderzijds 'spiersymptomen' (spierzwakte, inspanningsintolerantie, snelle vermoeibaarheid, spierkrampen). Dit overzichtsartikel beschrijft de structuur, functie en onderlinge interactie van de genoemde ECM moleculen, en wijst zo naar de gemeenschappelijke moleculaire achtergrond van deze aandoeningen. Dokters en onderzoekers die zich bezighouden met deze spierziekten en erfelijke bindweefselziekten moeten zich hier bewust van zijn. Het brede scala aan klachten van deze patiënten vraagt om een multidisciplinaire benadering. Daarnaast is het voor neurologen van belang om bij patiënten met neuromusculaire klachten te letten op gewrichtshypermobiliteit, omdat dit kan bijdragen aan het stellen van een specifieke diagnose.

Dit overzichtsartikel heeft zo de basis gelegd voor het verdere onderzoek in dit proefschrift.

Deel II: Neuromusculaire kenmerken van Ehlers-Danlos syndroom
Deel IIA: Klinische beoordeling van Ehlers-Danlos syndroom patiënten

Deel IIA begint met een klinische beschrijving van drie individuele EDS patiënten met bijzondere neuromusculaire symptomen. Daarna worden de resultaten van het klinische onderzoek in 40 EDS patiënten en van het vragenlijst onderzoek in 273 EDS patiënten beschreven.

Hoofdstuk 3: Eerste klinische observaties in Ehlers-Danlos syndroom

De eerste patiënte is een 30-jarige vrouw met het hypermobiele type EDS die meerdere drukneuropathieën heeft doorgemaakt: van de n.axillaris, van de plexus brachialis, van de n.peroneus, en van de n.ischiadicus. Deze drukneuropathieën werden allen bevestigd met neurofysiologisch onderzoek, en genetisch onderzoek naar een erfelijke drukneuropathie liet geen PMP22 mutatie zien. Het pathofysiologisch mechanisme van herhaaldelijke drukneuropathieën bij een patiënt met EDS lijkt voor de hand te liggen: door abnormale rek of druk ten gevolge van de hypermobiliteit zouden zenuwen kunnen beschadigen. Het zou echter ook kunnen zijn dat het bindweefsel in en om de perifere zenuwen minder stevig is, waardoor de zenuwen kwetsbaarder zijn voor abnormale druk of rek. Dokters die betrokken zijn bij de zorg voor EDS patiënten zouden hier rekening mee moeten houden.

De tweede patiënt is een 16-jarige jongeman met het kyfoscoliotische type van EDS veroorzaakt door een homozygote deletie van *PLOD1* (lysine hydroxylase 1; 1p36.3-p36.2), met proximale en distale spierzwakte, en hypotonie. Spierzwakte en hypotonie was in dit type EDS wel beschreven in de neonatale fase, maar niet op latere leeftijd. Bij aanvullend onderzoek werden aanwijzingen gevonden voor een myopathie en een lichte axonale polyneuropathie, die waarschijnlijk beiden hebben bijgedragen aan de spierzwakte. Deze kennis

is van belang voor de tijdige herkenning van klachten en voor het opzetten van revalidatie-programma's voor patiënten met dit type EDS.

De derde patiënt is een 50-jarige vrouw met het TNX-deficiënte type EDS, die verwezen werd met ernstige distale spierzwakte, atrofie van de spieren in haar handen en contracturen. Ze had eveneens een gegeneraliseerde hypermobiliteit. Ze was jaren geleden ook al eens verwezen naar de polikliniek neurologie in verband met deze klachten, en het spierbiopt was destijds normaal. Het tweede spierbiopt uit de m.vastus lateralis liet evenmin myopathische afwijkingen zien, maar wel een licht verminderde aankleuring van het endomysium met collageen VI antilichamen. De combinatie van spierzwakte, hypermobiliteit en contracturen komt ook voor bij collageen VI myopathieën, en interactie tussen collageen VI en TNX is belangrijk voor een normale functie van deze moleculen. De beschrijving van deze patiënte illustreert het klinische en moleculaire continuüm van de erfelijke bindweefselziekten en bepaalde spierziekten.

Hoofdstuk 4: Systematische klinische observationele studie naar neuromusculaire kenmerken van Ehlers-Danlos syndroom

De verscheidenheid aan klachten in de drie patiënten beschreven in *Hoofdstuk* 3 heeft bijgedragen aan de opzet van de studie beschreven in *Hoofdstuk 4*. Dit onderzoek bestaat uit: een korte gestandaardiseerde vragenlijst, lichamelijk onderzoek, zenuwgeleidingsonderzoek en elektromyografisch onderzoek (EMG), spierecho en spierbiopsie in 40 EDS patiënten van het vaattype, het klassieke type, het TNX-deficiënte type, en het hypermobiele type veroorzaakt door haploinsufficiëntie van *TNXB* (n = 10 van ieder type).

Het merendeel van de patiënten meldde de volgende klachten: spierzwakte, spierpijn, en snelle vermoeibaarheid (respectievelijk 65%, 73%, en 60%). Lichte tot matige spierzwakte (85%) en vermindering van de vibratiezin (60%) kwam vaak voor. Zenuwgeleidingsonderzoek liet een axonale polyneuropathie zien in vijf patiënten (13%), zonder dat hiervoor een metabole oorzaak werd gevonden. Naald EMG liet overwegend myopatische kenmerken zien in 23% van de patiënten, en een gemengd neurogeen – myopathisch patroon in 53% van de patiënten. Spierecho liet een verhoogde echo intensiteit en spieratrofie zien in de helft van de patiënten (50% en 48%). In vijf van de 18 spierbiopten werden lichte myopatische afwijkingen gevonden (28%). Bij aanvullend elektronmicroscopisch onderzoek van het spierbiopt werd gezien dat de dichtheid en de lengte van de collageen vezels in de EDS patiënten verminderd was.
Gemiddeld hadden de patiënten met het hypermobiele type EDS ten gevolge van *TNXB* haploinsufficiëntie (en verminderde aanwezigheid van TNX in serum) de minste klachten en symptomen. Dit kan wijzen op een dosis-effect relatie van de resterende hoeveelheid TNX (en daarmee de ernst van de ECM stoornis) en de ernst van het neuromusculaire fenotype. Verder vonden we in een deel van de patiënten ook aanwijzingen dat niet alleen de functie van spieren gestoord was, maar ook die van de perifere zenuwen.

Hoofdstuk 5 en 6: Vragenlijstonderzoek naar vermoeidheid en pijn in Ehlers-Danlos syndroom
Om te kunnen beoordelen hoe vaak pijn en vermoeidheid voorkomen bij patiënten met EDS, en om de invloed van deze klachten op het dagelijks leven te kunnen beoordelen, is vervolgens een schriftelijk vragenlijst onderzoek verricht onder 273 leden van de EDS patiëntenvereniging.

Meer dan driekwart van de EDS patiënten (77%) had last van ernstige vermoeidheid. Patiënten die erg vermoeid waren, hadden ook meer beperkingen in het dagelijks leven en meer psychische klachten. De vijf mogelijke determinanten die betrokken zijn in vermoeidheid bij EDS waren slaapstoornissen, concentratieproblemen, sociaal functioneren, de indruk de vermoeidheid zelf te kunnen beïnvloeden en ernst van de pijn. Omdat dit onderzoek een cross-sectionele opzet had, is niet bepaald of deze determinanten oorzaak of gevolg van de ernstige vermoeidheid zijn. Aan de hand van eerder longitudinaal onderzoek valt dit wel te beredeneren (zie ook *Figuur 2* in *Hoofdstuk 13*). Zo zouden deze determinanten een beginpunt kunnen vormen voor cognitieve gedragstherapie in EDS.

Omdat pijn een van de belangrijke determinanten van vermoeidheid was, is hier in het vragenlijstonderzoek verder naar gekeken (*Hoofdstuk 6*). Hieruit kwam naar voren dat chronische pijn veel voorkomt bij patiënten met EDS (90%) en dat het vaak gepaard gaat met het gebruik van pijnstillers. Verder kwam pijn vaker voor en was het ernstiger in het hypermobiele type EDS. De ernst van de pijn was gecorreleerd aan de hypermobiliteit, dislocaties, eerdere operaties, en aan slechte slaapkwaliteit. Pijn bleek ook bij te dragen aan functionele beperkingen in het dagelijkse leven, onafhankelijk van het nivo van vermoeidheid. Deze resultaten laten zien dat behandeling van pijn een belangrijk onderdeel van de symptomatische aanpak van EDS zou moeten zijn.

Deel IIB: Kwantitatief spierfunctie onderzoek van tenascine-X-deficiënte Ehlers-Danlos syndroom patiënten en tenascine-XB knockout muizen

De studies in deel IIB richten zich op het pathofysiologisch mechanisme van spierzwakte in EDS. Door dit onderzoek is duidelijker geworden hoe spierzwakte in EDS ontstaat en komen we meer te weten over de rol van de ECM in het functioneren van spieren. Deze onderzoeken zijn uitgevoerd in TNX-deficiënte EDS patiënten en *Tnxb* knockout (KO) muizen, een diermodel van EDS. *Tabel 1* in *Hoofdstuk 9* en *Tabel 1* van *Hoofdstuk 10* geven een overzicht van de gebruikte concepten, en in *Box 3* van *Hoofdstuk 13* wordt een overzicht gegeven van de verschillende onderzoeksmethoden. In *Tabel 1* van *Hoofdstuk 13* worden de resultaten van deze studies samengevat en met elkaar in verband gebracht.

Hoofdstuk 7 Kwantitatief spierfunctie onderzoek van twee tenascine-X-deficiënte Ehlers-Danlos syndroom patiënten

In dit hoofdstuk zijn de resultaten van kwantitatieve spierfunctie onderzoeken in twee TNX-deficiënte EDS patiënten beschreven. De krachtmomenten (= maat voor het rotatie-effect van een kracht; de grootte van een moment is bepaald als het product van kracht en krachtarm) werden gemeten bij relatief lange spierlengte. De TNX-deficiënte patiënten hadden verminderde maximale krachtmomenten bij vrijwillige contractie, relatief hoge twitch-momenten (krachtmomenten na enkelvoudige stimuli), een normaal interval tussen elektrische stimulatie en de krachtsopbouw, een snellere krachtsopbouw, en een normale relaxatie.

Het is onwaarschijnlijk dat deze afwijkingen zijn toe te schrijven aan verminderde fysieke activiteit of spieratrofie. Bij lichamelijk onderzoek, spierecho en spierbiopsie van deze patiënten werden namelijk geen tekenen gevonden van atrofie, en de twee patiënten hadden overeenkomstige afwijkingen, terwijl de ene patiënt fysiek veel actiever was dan de andere. De resultaten kunnen ook niet geheel verklaard worden door veranderingen van de krachtsoverdracht via de pees door een hogere compliantie (= de mate van elasticiteit) van die pees. Dan zou immers verwacht worden dat het krachtmoment dat opgebouwd wordt lager is en dat de relaxatie vertraagd zou zijn, en dat werd niet gevonden. Het zou daarentegen mogelijk kunnen zijn dat de TNX deficiëntie de stijfheid van de myofasciale verbindingen (gevormd door het bindweefsel in en om de spieren en rond de vaat-zenuwstreng) vermindert, en dat deze daardoor minder goed in staat zijn om onderling krachten over te dragen. Dit is in de volgende studies verder onderzocht (*Hoofdstuk 9* en *10*).

Hoofdstuk 8: Gestandaardiseerde neuromusculaire beoordeling van de tenascine-XB knockout muizen

Voorafgaand aan het kwantitatieve spierfunctie onderzoek in het muismodel van EDS (*Hoofdstuk 9*) is een aantal onderzoeken verricht om na te gaan of en zo ja welke neuromusculaire symptomen deze muizen hebben (*Hoofdstuk 8*). Er werden twee gestandaardiseerde functionele krachttesten gedaan ('hangtime' en 'paw-fall-through test'). Daarnaast werd een langdurige meting van de spontane bewegingen van deze muizen verricht, en zijn spier- en zenuwbiopten genomen.

Bij de functionele testen werd een lichte spierzwakte van de *Tnxb* KO muizen vastgesteld. Dit ging niet gepaard met een vermindering van de spontane motoriek in een kooi gedurende de observatie van een week. De spierbiopten lieten lichte myopatische veranderingen zien, en bij RNA micro array analyse van spier werd gevonden dat een aantal genen betrokken bij de aanmaak en afbraak van ECM eiwitten sterker tot expressie komt. Een n.ischiadicus biopsie liet een vermindering van de dichtheid en lengte van de collageenvezels tussen de zenuwvezels zien, met tekenen van de- en regeneratie en kleinere diameters van de gemyeliniseerde vezels.

Deze bevindingen sluiten aan bij de bevindingen in TNX-deficiënte EDS patiënten (*Hoofdstuk 4*), bij wie ook zowel myopathische als neuropathische afwijkingen werden gevonden. Deze *Tnxb* KO muis lijkt dus een goed model te vormen voor TNX-deficiënte vorm van EDS.

Hoofdstuk 9: Kwantitatief spierfunctie onderzoek van tenascine-XB knockout muizen
Het doel van de studie beschreven in dit hoofdstuk is om vast te stellen of TNX deficiëntie inderdaad de stijfheid van de myofasciale verbindingen vermindert, zodat deze minder goed in staat zijn om krachten over te dragen. Het onderzoek bestaat uit twee delen: in het eerste deel worden intramusculaire aspecten van spierkracht onderzocht tijdens isometrische contracties van de maximaal vrijgeprepareerde m. gastrocnemius mediale (GM)(*Serie A*); in het tweede deel worden juist de intermusculaire aspecten van spierkracht gemeten aan de hand van kracht gemeten in de m. triceps surae (TS), de m.extensor digitorum longus (EDL); de m.extensor hallucis longus (EHL); en de m.tibialis anterior (TA) – dit terwijl de anatomische verhoudingen tussen deze spieren intact zijn (*Serie B*). Op deze manier kunnen de interacties tussen de genoemde spieren gemeten worden.

Bij *serie A* werden alleen verschillen gevonden bij korte spierlengte (optimum lengte - 4, - 3.5, - 3 mm): verminderde genormaliseerde actieve isometrische kracht, een langer interval tussen elektrische stimulus en het bereiken van 2% van de maximale kracht, en vertraagde relaxatie in de *Tnxb* KO muis. Deze resultaten worden veroorzaakt door veranderingen van de serie elastische component binnen het maximaal vrijgeprepareerde GM spier-peescomplex. Het is niet vreemd dat dit alleen gevonden werd bij korte spierlengte, omdat juist dan meer 'slack' opgenomen moet worden voordat de serie elastische component opgespannen is en kracht kan overdragen. Verder is het opvallend dat de genormaliseerde actieve isometrische kracht bij optimum spierlengte en daarboven normaal was, terwijl bij de twee TNX-deficiënte EDS patiënten in de pilot studie het krachtmoment wel verminderd was bij een vergelijkbare spierlengte. Dit verschil tussen kracht na elektrische stimulatie bij muizen en het krachtmoment tijdens vrijwillige contractie bij patiënten roept de vraag op of een verminderding van de activatie capaciteit wellicht bijdraagt aan de spierzwakte bij EDS patiënten.

De tweede serie experimenten in Tnxb KO muizen (*Serie B*) is gericht op de verandering van de serie elastische component tussen de spieren. Hierbij zijn ook alleen verschillen gevonden bij korte spierlengte. Normaal neemt de distal actieve kracht in de agonist (respectievelijk TA+EHL en TS) af als de antagonist langer wordt (respectievelijk TS en TA+EHL). Dit komt doordat er tussen spieren onderling kracht wordt overgedragen; dit wordt myofasciale krachtstransmissie genoemd. De TNX deficiëntie beperkt deze antagonist-lengte afhankelijke afname in actieve kracht van de agonist. Dit geeft aan dat de myofasciale krachtstransmissie verminderd is in de *Tnxb* KO muizen, waarschijnlijk ten gevolge van de

toegenomen compliantie van het bindweefsel rondom en tussen de individuele spieren. Ook de netto epimusculaire myofasciale krachtsoverdracht was verminderd in de *Tnxb* KO muizen (gemeten als verschil van kracht tussen de proximale en distale pezen van de EDL). Blijkbaar zijn de TNX-deficiënte spieren minder goed in staat om onderling krachten over te dragen, en functioneren ze meer zelfstandig. Dit kan van invloed zijn op de onderlinge afstemming van spiercontracties in complexe fysiologische omstandigheden, waardoor bewegingen minder efficiënt verlopen.

Hoofdstuk 10: Kwantitatief spierfunctie onderzoek van tenascine-X-deficiënte Ehlers-Danlos syndroom patiënten

De eerste stap om de bovenbeschreven resultaten te verifiëren in patiënten is om te kijken naar knie extensie in TNX-deficiënte patiënten bij relatief korte spierlengte, en om de activatie capaciteit te meten.

Dit is gedaan in zeven TNX-deficiënte EDS patiënten, met meting van de isometrische vrijwillige en elektrisch gestimuleerde contracties (van zowel knie extensie als knie flexie) bij verschillende spierlengtes (30°, 60° en 90° knie flexie) (*Hoofdstuk 10*).[23] De mate van fysieke activiteit verschilde niet tussen de patiënten en controle proefpersonen.

De belangrijkste bevindingen van deze studie zijn: 1) TNX-deficiënte EDS patiënten hadden een verminderd maximaal krachtmoment van knie extensoren bij alle spierlengtes; 2) de genormaliseerde krachtmomenten waren niet verschillend tussen patiënten en controles (genormaliseerd naar het hoogste krachtmoment bij 60%); 3) het interval tussen elektrische stimulus en opbouw van kracht was verlengd met name bij korte spierlengte; en 4) EDS patiënten hadden een verminderde vrijwillige activatie met name bij korte spierlengte.

Samen laten deze resultaten zien dat het isometrische vrijwillige maximale krachtmoment verminderd is in EDS, en bevestigen daarmee de bevindingen van de pilot studie in twee TNX-deficiënte EDS patiënten (*Hoofdstuk 7*). De verlenging van het interval tussen elektrische stimulus en de opbouw van kracht en de verminderde centrale activatie bij korte spierlengte wijzen in de richting van een toegenomen compliantie van de serie elastische component van het spier-peescomplex. Echter, dit heeft blijkbaar niet geleid tot duidelijke vermindering van het genormaliseerde krachtmoment bij korte spierlengte (ook al zouden er eigenlijk meer meetpunten nodig zijn om hier een meer definitieve uitspraak over te doen). Verder geven de resultaten aan dat een belangrijk deel van de spierzwakte verklaard kan worden door een onvermogen om de spieren maximaal aan te spannen.

Deel III: Neuromusculaire kenmerken van Marfan syndroom

Klinische evaluatie van Marfan syndroom patienten

Om na te gaan of de bevindingen beschreven in deel IIA specifiek zijn voor EDS, of ook gevonden kunnen worden bij andere erfelijke bindweefselaandoeningen (zoals beschreven

in het overzichtsartikel in *Hoofdstuk 2)*, is tenslotte gekeken naar het voorkomen van neuromusculaire symptomen in Marfan syndroom.

Hoofdstuk 11: Systematische klinische observationele studie naar neuromusculaire kenmerken van Marfan syndroom

Het onderzoek naar de neuromusculaire symptomen van Marfan syndroom bestaat uit een gestandaardiseerde vragenlijst, neurologisch onderzoek, zenuwgeleidingsonderzoek, naald EMG, laboratorium onderzoek en een spierbiopsie. Verder is gekeken naar de aanwezigheid van durale ectasie en meningospinale cysten aan de hand van eerder verricht beeldvormend onderzoek van de lumbosacrale wervelkolom.

Het bleek dat diverse neuromusculaire symptomen vaker voorkomen bij patiënten met Marfan syndroom dan bij controle proefpersonen (spierzwakte, spierpijn, snelle vermoeibaarheid, beperkte loopafstand). De vier oudste patiënten (> 50 jaar) rapporteerden spierzwakte en hadden functionele beperkingen. Vijf patiënten hadden een lichte vermindering van de vibratiezin. Het zenuwgeleidingsonderzoek liet aanwijzingen zien voor een axonale polyneuropathie bij vier patiënten, en bij elektromyografie werd bij alle patiënten een gemengd myopathisch – neurogeen patroon gezien. Het klinisch neurofysiologisch onderzoek liet tevens aanwijzingen voor radiculopathie op één of meer lumbosacrale nivo's, welke gerelateerd leken aan de durale ectasieën en spinale meningeale cystes bij beeldvormend onderzoek. De spierecho liet bij meer dan de helft van de patiënten een toegenomen echo intensiteit en spieratrofie zien. Een spierbiopt kon slecht bij twee patiënten verricht worden; in één van hen liet dit myopathische afwijkingen zien.

Samengevat had het merendeel van de Marfan patiënten neuromusculaire symptomen met bij aanvullend onderzoek een lichte myopathie of polyneuropathie of beiden, met daarnaast aanwijzingen voor lumbosacrale radiculopathie. De oudere patiënten hadden de meeste klachten en symptomen.

Hoofdstuk 12: Symptomen van durale ectasie en/of spinale meningeale cysten

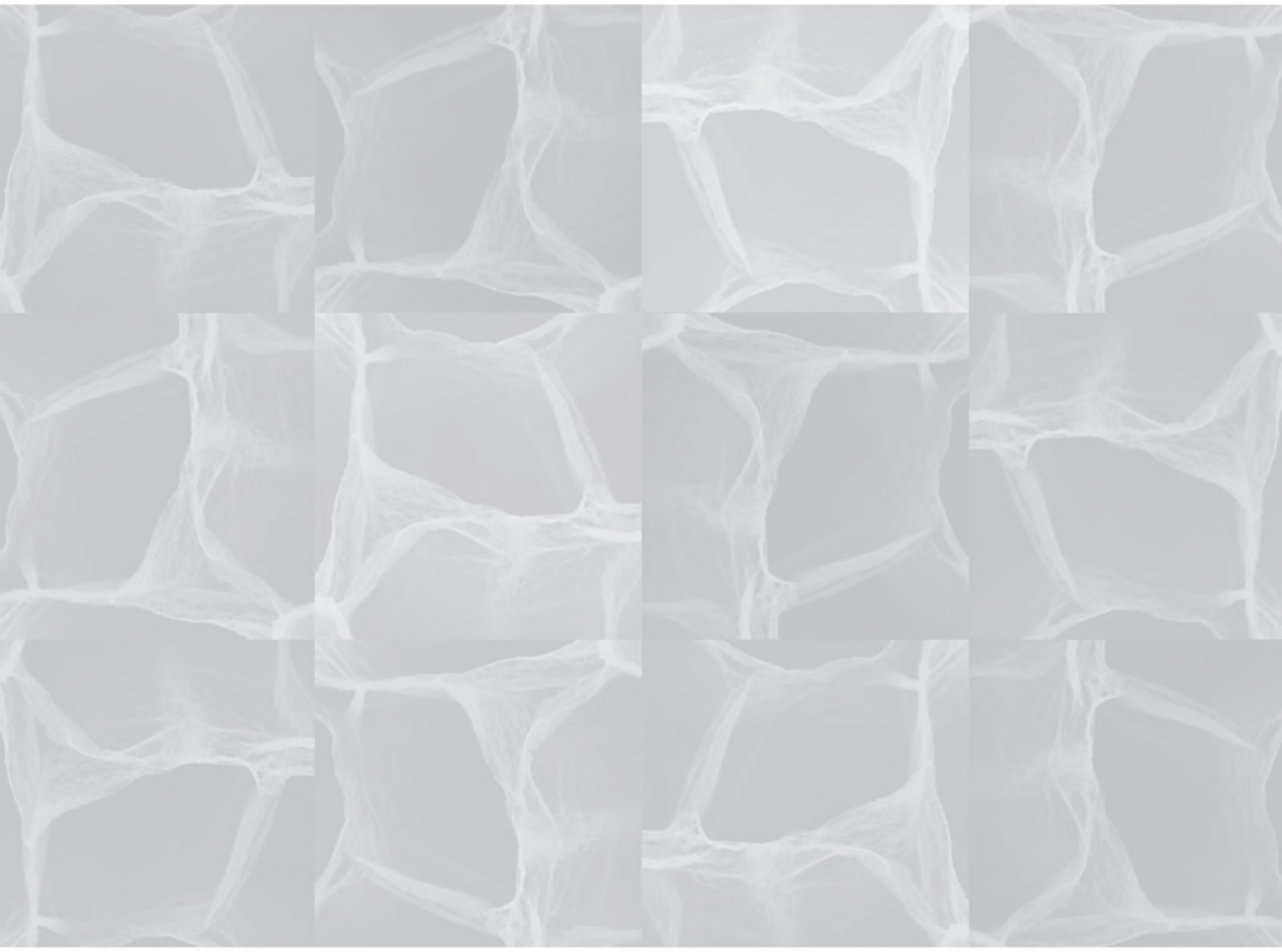
Omdat aanwijzingen voor lumbosacrale radiculopathie niet zijn aangetroffen bij patiënten met EDS is hier in drie Marfan patiënten uitgebreider naar gekeken. Durale ectasieën komen voor bij 90% van de volwassen Marfan patiënten en hebben de neiging om groter te worden bij het ouder worden. De durale ectasie ontstaat waarschijnlijk door de voortdurende hydrostatische druk van de liquor op het slappere dura weefsel. Omdat de dura slapper is, wordt er in het algemeen van uitgegaan dat deze geen radiculaire compressie kan veroorzaken. Benige afwijkingen aan de wervelkolom zouden wel kunnen bijdragen aan het ontstaan van radiculopathie bij Marfan syndroom.

In dit hoofdstuk worden drie patiënten met matige tot ernstige radiculopathie beschreven; de patiënten presenteerden zich met pijn, spierzwakte en –atrofie, en gevoels-

stoornissen. Beeldvormend onderzoek (MRI van de lumbosacrale wervelkolom) liet een nauw contact tussen de zenuwwortels en de durale ectasia en / of spinale meningeale cyste zien, waarbij de zenuwwortels zijn afgeplat en niet langer omgeven worden door vetweefsel. Meest waarschijnlijk werd de radiculaire prikkeling veroorzaakt door benige en durale compressie bij (kyfo)scoliose en durale ectasie met spinale meningeale cysten. Deze casus laten zien dat Marfan syndroom gepaard kan gaan met diverse neurologische symptomen, met name bij de oudere patiënten.

Box 7 in *Hoofdstuk 13* geeft een puntsgewijze samenvatting van de belangrijkste conclusies van dit onderzoek en aanwijzingen voor toekomstig onderzoek.

Appendix



Abbrevations

EDS	Ehlers-Danlos syndrome
ICTD	Inherited connective tissue disorder
ECM	Extracellular matrix
UCMD	Ullrich Congenital Muscular Dystrophy
TNX	Tenascin-X
MRC	Medical Research Council
KO	Knockout
WT	Wild type
DGC	Dystrophin glycoprotein complex
MDC	Congenital muscular dystrophy
LGMD	Limb girdle muscular dystrophy
WWS	Walker-Warburg syndrome
MEB	Muscle-eye-brain disease
FCMD	Fukuyama congenital muscular dystrophy
SJS	Schwartz-Jampel syndrome
SHS	Silverman Handmaker syndrome
OI	Osteogenesis imperfecta
MED	Multiple epiphyseal dysplasia
TGF-β	Transforming growth factor β
CCA	Congenital contractural arachnodactyly
LH1	Lysyl hydroxylase 1
CK	Creatine kinase
NCV	Nerve conduction velocity
NCS	Nerve conduction studies
CMAP	Compound muscle action potential
MUAP	Motor unit action potential
SNAP	Sensory nerve action potential
T/A	Turns / Amplitude analysis
EI	Echo intensity
EM	Electronmicroscopy
SD	Standard deviation
Clas	Classical type Ehlers-Danlos syndrome
Vasc	Vascular type Ehlers-Danlos syndrome
TNXd	Tenascin-X-deficient type Ehlers-Danlos syndrome
TNXh	Haploinsufficiency of *TNXB*, associated with hypermobility type Ehlers-Danlos syndrome
Hyper	Hypermobility type Ehlers-Danlos syndrome
CIS	Checklist Individual Strength
SIP	Sickness Impact Profile
SF-36	ShortForm-36
BDI-pc	Beck Depression Inventory
SCL-90	Symptom Check List
SES	Self Efficacy Scale

VAS	Visual analogous scale
PFT 1 min	Number of paw fall through events in 1 minute
HT dur	Hang time duration in sec
MMP	Matrixmetalloproteinases
IPAQ	International Physical Activity Questionnaire
TA	Tibial anterior muscle
GM	Medial gastrocnemius muscle
EHL	Extensor hallucis longus muscle
EDL	Extensor digitorum brevis muscle
TS	Triceps surae muscle
ℓ_o (mm)	Optimum length
Fmp	Passive force
Fma	Active muscle force
%Fma	Normalized active force
Fmt	Total force
F_o	Optimum force
peakFma	Active peak force
%MRR	Normalized maximal rate of relaxation
%MRFR	Normalized maximal rate of force rise
MET	Metabolic equivalent
MVT	Maximal voluntary torque
nMVT	Normalized MVT
VA	Voluntary activation capacity en voluntary activation index
MTC	Maximal torque capacity
MRTD	Maximal rate of torque development
nMRTD	Normalized MRTD
tMRTD	Time to reach MRTD

List of publications

Mahler EA, Blom M, Voermans NC, van Engelen BG, van Riel PL, Vonk MC. Rituximab treatment in patients with refractory inflammatory myopathies. Rheumatology (Oxford). 2011 May 13. [Epub ahead of print]

Geurts AC, Boonstra TA, Voermans NC, Diender MG, Weerdesteyn V, Bloem BR. Assessment of postural asymmetry in mild to moderate Parkinson's disease. Gait Posture. 2011;33:143-5.

Voermans NC, Knoop H, van Engelen BG. High frequency of neuropathic pain in Ehlers-Danlos syndrome: an association with axonal polyneuropathy and compression neuropathy? J Pain Symptom Manage. 2011;41:e4-6.

Voermans NC, Knoop H. Both pain and fatigue are important possible determinants of disability in patients with the Ehlers-Danlos syndrome hypermobility type. Disabil Rehabil. 2011;33:706-7.

Voermans NC, Verrijp K, Eshuis L, Balemans MC, Egging D, Sterrenburg E, van Rooij IA, van der Laak JA, Schalkwijk J, M van der Maarel S, Lammens M, van Engelen BG. Mild Muscular Features in Tenascin-X Knockout Mice, A Model of Ehlers-Danlos Syndrome. Connect Tissue Res. 2011 Mar 15. [Epub ahead of print]

Voermans NC, Knoop H, Bleijenberg G, van Engelen BG. Fatigue is associated with muscle weakness in Ehlers-Danlos syndrome: an explorative study. Physiotherapy 2011 Jun;97:170-4.

Voermans N, van Engelen B. Quality of Life in the Classic and Hypermobility Types of Ehlers-Danlos Syndrome. Ann Neurol 2010;67:146-7.

Besselink-Lobanova A, Maandag NJ, Voermans NC, van der Heijden HF, van der Hoeven JG, Heunks LM. Trachea rupture in tenascin-X-deficient type Ehlers-Danlos syndrome. Anesthesiology. 2010;113:746-9.

Voermans NC, Knoop H, Bleijenberg G, van Engelen BG. Pain in Ehlers-Danlos syndrome is common, severe, and associated with fatigue and functional impairment. J Pain Symptom Manage. 2010;40:370-8.

Huijing PA, Voermans NC, Baan GC, Busé TE, Van Engelen BG, de Haan A. Muscle characteristics and altered myofascial force transmission in tenascin-X deficient mice, a mouse model of Ehlers-Danlos syndrome. J Appl Physiol. 2010;109:986-95.

Voermans NC, Knoop H, van de Kamp N, Hamel BC, Bleijenberg G, van Engelen BG. Fatigue Is a Frequent and Clinically Relevant Problem in Ehlers-Danlos Syndrome. Semin Arthritis Rheum. 2010;40:267-74.

Voermans NC, Guillard M, Doedée R, Lammens M, Huizing M, Padberg GW, Wevers RA, van Engelen BG, Lefeber DJ. Clinical features, lectin staining, and a novel GNE frameshift mutation in hereditary inclusion body myopathy. Clin Neuropathol. 2010;29:71-7.

Voermans NC, Hosman AJ, van Alfen N, Bartels RH, de Kleuver M, op den Akker JW, van Engelen BG. Radicular dysfunction due to spinal deformities in Marfan syndrome at older age: three case reports. Eur J Med Genet. 2010;53:35-9.

Voermans NC. Dural ectasia in Marfan syndrome. Neurology. 2009;73:2047.

Voermans NC, van Alfen N, Pillen S, Lammens M, Schalkwijk J, Zwarts MJ, van Rooij IA, Hamel BC, van Engelen BG. Neuromuscular involvement in various types of Ehlers-Danlos syndrome. Ann Neurol. 2009;65:687-97.

Voermans NC, Dijk KG, Bos MM, Geus-Oei LF, Verrips A, Lindert EJ. Postural headache in marfan syndrome associated with spinal cysts and liquor hypotension. Neuropediatrics. 2009;40:201-4.

Voermans NC, Bönnemann CG, Lammens M, van Engelen BG, Hamel BC. Myopathy and polyneuropathy in an adolescent with the kyphoscoliotic type of Ehlers-Danlos syndrome. Am J Med Genet A. 2009;149A:2311-6.

Voermans N, Timmermans J, van Alfen N, Pillen S, op den Akker J, Lammens M, Zwarts MJ, van Rooij IA, Hamel BC, van Engelen BG. Neuromuscular features in Marfan syndrome. Clin Genet. 2009;76:25-37.

Voermans NC, Bonnemann CG, Hamel BC, Jungbluth H, van Engelen BG. Joint hypermobility as a distinctive feature in the differential diagnosis of myopathies. J Neurol. 2009;256:13-27.

Horlings CG, Küng UM, van Engelen BG, Voermans NC, Hengstman GJ, van der Kooi AJ, Bloem BR, Allum JH. Balance control in patients with distal versus proximal muscle weakness. Neuroscience. 2009;164:1876-86.

Voermans NC, Minnema M, Lammens M, Schelhaas HJ, Kooi AV, Lokhorst HM, van Engelen BG. Sporadic late-onset nemaline myopathy effectively treated by melphalan and stem cell transplant. Neurology. 2008;71:532-4.

Voermans NC, Bönnemann CG, Huijing PA, Hamel BC, van Kuppevelt TH, de Haan A, Schalkwijk J, van Engelen BG, Jenniskens GJ. Clinical and molecular overlap between myopathies and inherited connective tissue diseases. Neuromuscul Disord. 2008;18:843-56.

Voermans NC, van Engelen BG. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). Neuromuscul Disord. 2008;18:906.

Pieterse AJ, Voermans NC, Tuinenga HS, van Engelen BG. Computer-aided visualization of muscle weakness distribution. J Neurol. 2008;255:1670-8.
Voermans NC, van Alfen N, Drost G, Ginjaar HB, Willemsen MA. Thought ripples on muscle waves: recognition of rippling muscle disease. Neuropediatrics. 2008;39:116-8.
Voermans NC, Pillen S, de Jong EM, Creemers MC, Lammens M, van Alfen N. Morphea profunda presenting as a neuromuscular mimic. J Clin Neuromuscul Dis. 2008;9:407-14.
Voermans N, van Engelen B. Cervical myelopathy caused by retrograde intraneural dissection of anesthetic solution. Muscle Nerve. 2008;37:546-7.
Voermans NC, Altenburg TM, Hamel BC, de Haan A, van Engelen BG. Reduced quantitative muscle function in tenascin-X deficient Ehlers-Danlos patients. Neuromuscul Disord. 2007;17:597-602.
Voermans NC, Jenniskens GJ, Hamel BC, Schalkwijk J, Guicheney P, van Engelen BG. Ehlers-Danlos syndrome due to tenascin-X deficiency: muscle weakness and contractures support overlap with collagen VI myopathies. Am J Med Genet A. 2007;143A:2215-9.
Voermans NC, Schutte PJ, Bloem BR. Hydrocephalus induced chorea. J Neurol Neurosurg Psychiatry. 2007;78:1284-5.
Molema MM, Dekker MC, Voermans NC, van Engelen BG, Aarnoutse RE. Caffeine and muscle cramps: a stimulating connection. Am J Med. 2007;120:e1-2.
Voermans NC, Snijders AH, Schoon Y, Bloem BR. Why old people fall (and how to stop them). Pract Neurol. 2007;7:158-71.
Visser JE, Voermans NC, Oude Nijhuis LB, van der Eijk M, Nijk R, Munneke M, Bloem BR. Quantification of trunk rotations during turning and walking in Parkinson's disease. Clin Neurophysiol. 2007;118:1602-6.
Voermans NC, Schelhaas HJ, Munneke M, Zwarts MJ. Dissociated small hand muscle atrophy in aging: the 'senile hand' is a split hand. Eur J Neurol. 2006;13:1381-4.
Voermans NC, van Alfen N, Tolboom JJ, Koetsveld AC, Sie LT. Pediatric median neuropathy due to pruritus in Alagille syndrome. Pediatr Neurol. 2006;35:216-9.
Voermans NC, Bloem BR, Janssens G, Vogel WV, Sie LT. Secondary parkinsonism in childhood: A rare complication after radiotherapy. Pediatr Neurol. 2006;34:495-8.
Voermans NC, Koetsveld AC, Zwarts MJ. Segmental overlap: foot drop in S1 radiculopathy. Acta Neurochir (Wien). 2006;148:809-13.
Voermans NC, van Engelen BG, Kluijtmans LA, Stikkelbroeck NM, Hermus AR. Rhabdomyolysis caused by an inherited metabolic disease: very long-chain acyl-CoA dehydrogenase deficiency. Am J Med. 2006;119:176-9.
Voermans NC, Crul BJ, de Bondt B, Zwarts MJ, van Engelen BG. Permanent loss of cervical spinal cord function associated with the posterior approach. Anesth Analg. 2006;102:330-1.
Voermans NC, Drost G, van Kampen A, Gabreëls-Festen AA, Lammens M, Hamel BC, Schalkwijk J, van Engelen BG. Recurrent neuropathy associated with Ehlers-Danlos syndrome. J Neurol. 2006;253:670-1.
Voermans NC, Poels PJ, Kluijtmans LA, van Engelen BG. The effect of dantrolene sodium in Very Long Chain Acyl-CoA Dehydrogenase Deficiency. Neuromuscul Disord. 2005;15:844-6.
Voermans NC, Zwarts MJ, van Laar T, Tijssen MA, Bloem BR. Fallacious falls. J Neurol. 2005;252:1271-3.
Voermans NC, Lammens M, Wevers RA, Hermus AR, van Engelen BG. Statin-disclosed acid maltase deficiency. J Intern Med. 2005;258:196-7.
Voermans NC, Vaneker M, Hengstman GJ, ter Laak HJ, Zimmerman C, Schelhaas HJ, Zwarts MJ. Primary respiratory failure in inclusion body myositis. Neurology. 2004 Dec 14;63(11):2191-2.
Voermans NC, Petersson KM, Daudey L, Weber B, Van Spaendonck KP, Kremer HP, Fernández G. Interaction between the human hippocampus and the caudate nucleus during route recognition. Neuron. 2004;43:427-35.
Meulenbroek O, Petersson KM, Voermans N, Weber B, Fernández G. Age differences in neural correlates of route encoding and route recognition. Neuroimage. 2004;22:1503-14.

Publications in Dutch:

Perdok JM, van Dongen R, van Wijhe M, Voermans NC, Beems T, Staal MJ. Behandeling van centrale pijn en aangezichtspijn. Ned Tijdschr Geneeskd. 2009;153:538-42.

Voermans NC, Jacobs B, van de Laar FA, van Sorge-Greve AH, van Engelen BG, Vos PE. Licht traumatisch schedelhersenletsel bij een oudere patiënt met orale antistolling. Ned Tijdschr Geneeskd. 2009;153:130-5.

Voermans NC, Beems T, Van Alfen N, Drost G, Van Wijhe M, Staal MJ, Perdok JM, Van Dongen R. Motorcortexstimulatie bij persisterende centrale pijn en aangezichtspijn. Tijdschrift voor neurologie en neurochirurgie 2008;109:127-35.

Voermans NC, Schutte PJ, Bloem BR. Chorea veroorzaakt door hydrocefalus. Tijdschrift voor neurologie en neurochirurgie 2007;108:334-8.

Zonneveld AM, Hagenaars M, Voermans NC, Gelissen HP, Claassen JA. Levensbedreigend serotoninesyndroom na eenmalige toevoeging van een serotonineheropnameremmer aan een onderhoudsbehandeling met een monoamineoxidaseremmer. Ned Tijdschr Geneeskd. 2006;150:1081-4.

Erasmus CE, Voermans NC, Bloem BR, Janssens G, Vogel WV, Sie LT. Secundair parkinsonisme op de kinderleeftijd. Tijdschrift voor neurologie en neurochirurgie 2006;107:282-8.

Voermans NC, Zwarts MJ, Renier WO, Bloem BR. Epileptische aanvallen in het kraambed bij een patiënte met idiopathische gegeneraliseerde epilepsie. Ned Tijdschr Geneeskd. 2005;149:1406-11.

Van Hamont D, Voermans NC, Boerman RH, Cruysberg JR. Visuele hallucinaties bij intacte realiteitstoetsing: pseudohallucinaties. Tijdschrift voor neurologie en neurochirurgie 2005;106:262-8.

Voermans NC, Hermens FH, Van Engelen BG, Koetsveld AC. Neurologische complicaties bij de ziekte van Rendu-Osler-Weber. Tijdschrift voor neurologie en neurochirurgie 2005;106:163-9.

Voermans NC, Hart W, van Engelen BG. Klinisch denken en beslissen in de praktijk. Een 23-jarige vrouw met malaise, anorexie en gedragsveranderingen. Ned Tijdschr Geneeskd. 2004;148:1079-86.

Curriculum Vitae

Nicol Voermans was born in Zetten, the Netherlands in 1973. She attended the Heldring College in Zetten and finished her secondary education there (cum laude). She attended the Angelo State University in San Angelo, Texas in 1991-1992 as an exchange student and completed her freshman year with an A-average. She started medical school at the Radboud University Nijmegen in 1992, and performed an internship in Tel Aviv, Israel in 1997 and in Berekum, Ghana in 2000. She finished her medical studies in 2000 (cum laude). She also studied philosophy at the Radboud University Nijmegen between 1995-1998, and graduated in the field of philosophical anthropology (cum laude). Her final thesis was on the phenomenology of Merleau-Ponty and its relevance for the current medical practice. She started working as a resident at the department of Neurology of the Radboud University Nijmegen Medical Centre in 2000, and started her specialization in 2001. In 2002-2004 she performed a functional MRI study on implicit memory in patients with Huntington Disease at the FC Donders Centre for Neuroimaging in Nijmegen, for which she was awarded with the C.U. Ariëns Kappersprijs of the Dutch Neurology Association. In 2005, she was granted the AGIKO scholarship of the Netherlands Organisation for Scientific Research, which enabled her to perform the studies described in this thesis. She finished her specialization in 2008, after which she continued working as a neurologist at the same hospital. In 2009, she received a clinical neuromuscular fellowship of the Prinses Beatrix Foundation, which enabled her to learn more about the histopathological and genetic aspects of various neuromuscular disorders.

Dankwoord

Allereerst wil ik de patiënten die aan dit onderzoek hebben deelgenomen hartelijk bedanken. De Vereniging van Ehlers-Danlos patiënten en de Contacgroep Marfan Nederland dank ik voor hun medewerking en interesse. Ik hoop dat de resultaten zullen bijdragen aan een betere herkenning en erkenning van het brede scala aan symptomen van Ehlers-Danlos syndroom en Marfan syndroom. Het zou mooi zijn als deze studie een aanleiding vormt voor verder onderzoek naar behandelingsmogelijkheden.

Baziel, hartelijk dank voor alles wat ik van je geleerd heb tijdens dit promotietraject. Jij wilde de rol van bindweefsel in spier al een tijd nader onderzoeken, en wij hebben samen nagedacht over de opzet van dit onderzoek. Jij moedigde mij aan om een aantal case-reports te gaan schrijven om de kansen op een subsidie te verhogen, en in 2005 werd onze NWO AGIKO aanvraag inderdaad toegekend. In 2006 kon ik starten met de uitvoering van dit onderzoek. Jij hebt mij daarin veel ruimte gegeven. Jij introduceerde de tenascin-XB knockout muis, en nam me mee naar het IFKB en het NKCV. Je was zelf vaak druk, maar wel altijd op korte termijn beschikbare voor vragen. Je gaf goede inhoudelijke feedback op manuscripten, en nodigde me uit om te zoeken naar verbanden tussen de uitkomsten van de verschillende onderzoeken. In het begin van dit project liet je me een model van coaching zien, en ik vind het leuk dat ik in de loop van de jaren diverse stadia met je heb kunnen doorlopen. Ik kijk uit naar onze toekomstige samenwerking. Verder ben ik jou en Luki dankbaar voor jullie interesse in mijn leven buiten de neurologie, en voor jullie gastvrijheid voor mij en mijn gezin.

Ben, jij hebt vanaf het begin van dit project meegedacht over de te includeren patiënten en klinische onderzoeksmethoden. Hartelijk dank daarvoor, en voor het beoordelen van patiënten bij wie de diagnose onduidelijk was.

Arnold, jij hebt mij een heel andere methode van onderzoek van spierfunctie geleerd. Hartelijk dank voor je bereidheid om een andere patiëntencategorie en een nieuw diermodel te gaan onderzoeken. Bedankt dat je Karin hebt uitgenodigd om de vervolg onderzoeken in tenascine-X deficiënte patiënten te gaan verrichten; het was ook een groot plezier om met haar samen te werken. Tinelies, bedankt voor het uitvoeren van de experimenten in de muizen.

Peter en Guus, dank voor jullie enthousiasme om te gaan onderzoeken hoe de myofasciale krachtstransmissie veranderd is in TNX-deficiënte muizen. Het zijn deze experimenten die meer directe aanwijzingen hebben gegeven dat het bindweefsel in en tussen de spieren van belang is in de transmissie van kracht.

Martin, dank voor je inzet in de verschillende experimenten bij muizen en patiënten. Je was daarin steeds erg flexibel en meedenkend. Ook dank aan Kiek, Lilian, Jeroen, Ellen en Monique voor jullie bijdrage aan de experimenten bij de muizen.

Joost, David en Manon, ik vond het leuk om verder te werken aan het onderwerp waar jullie jaren geleden mee gestart zijn. Het is mooi dat we nu ook meer weten over de neuromusculaire kenmerken van tenascine-X deficiëntie. Hartelijk dank voor jullie hulp bij het oproepen van patiënten, bij het opzetten van de experimenten bij de muizen, en voor jullie feedback op de manuscripten.

Gijs en Hans, hartelijk dank voor de prettige samenwerking in het onderzoek naar vermoeidheid en pijn bij de Ehlers-Danlos patiënten. Het vragenlijst onderzoek vormt een mooie aanvulling op de overige studies. Hans, hartelijk dank voor je prettige begeleiding tijdens de statische analyses. Nicole en Rineke, dank voor jullie uitgebreide data invoer.

Janneke, dank voor de selectie van patiënten en je gastvrijheid op de Marfan polikliniek.

Nens, Sigrid, Machiel, en alle KNF-laboranten, hartelijk dank voor het enthousiasme waarmee jullie hebben meegewerkt aan dit project.

Carsten and Heinz, thank you for your nice cooperation; hopefully it will continue.

Guillen, Berry, and Karl Magnus, thanks for your help and enthusiasm in my first scientific experiences. I have learnt a lot at the F.C. Donders Centre, and I am very pleased that all the effort Guillen, and especially Karl-Magnus have put in coaching me throughout the analyses have resulted in a nice article and have contributed to my pleasure in performing subsequent scientific experiments.

George, hartelijk dank voor de ruimte die ik tijdens en na mijn opleiding heb kunnen nemen om aan dit en ander onderzoek te kunnen werken. De afdeling neurologie is bij uitstek een inspirerende onderzoeksomgeving.

Bas, dank voor de prettige samenwerking in het schrijven van een aantal case reports tijdens mijn opleiding. Ik heb daar veel van geleerd en jouw plezier in het schrijven werkte aanstekelijk. Ik hoop dat we in de toekomst vaker zullen samenwerken.

Mijn overige collega's van de (kinder)neurologie en klinische neurofysiologie; hartelijk dank voor de prettige samenwerking op allerlei vlakken. Ik heb veel geleerd tijdens mijn opleiding en vind het erg mooi om nu te kunnen bijdragen aan die opleiding.

Iris, wij kennen elkaar al jaren, en ik vond het erg leuk dat jij mee wilde werken aan drie artikelen in dit proefschrift. Ik vind het geweldig dat jij mijn paranimf bent. Bram, wat was het leuk om jou niet alleen als collega, maar ook als buurman te hebben. Hartelijk dank voor de gezelligheid over de schutting, en voor het oppassen op Marie. Ik vind het leuk dat jij nu met plezier in Groningen werkt, en dank dat je nu mijn paranimf bent.

Riky, jou wil ik graag van harte bedanken voor de goede zorgen voor onze kinderen. Dankzij jou kan ik met een gerust gevoel naar mijn werk gaan. Het is zo fijn om te weten dat ze in goede handen zijn. Ik hoop dat ik nog lang op je mag vertrouwen. Dank ook aan Monique, Emmy, Adua, Chris, Hanneke en Jo-Ann. Jullie doen geweldig werk! Marga, dankzij jouw inzet heb ik meer tijd voor datgene wat mij veel meer interesseert; hartelijk dank voor al je hulp.

Ingrid, dank voor je interesse in mijn werk en goede zorgen voor ons allen. Yvon, John en Mirjam, de band die op de Koninginnelaan is ontstaan blijft bestaan. Gerard en Ilse, dank voor jullie betrokkenheid bij dit en andere projecten. Janske, Arianne en Kitty, dank voor jullie mooie vriendschap en steun. Isa and Nicole, how wonderful that we have stayed in touch since San Angelo 1991-1992; thanks for your friendship. Elly en Maaike, dank voor de vriendschap en de prettige samenwerking tijdens onze opleiding. Wat leuk dat we nu patiënten naar elkaar kunnen verwijzen. Alide, dank voor je steun tijdens posterpresentaties en het overleg over diverse aspecten van onze promoties. Willemijn en Steven, ik vind het erg leuk dat we elkaar op de Driehuizerweg hebben leren kennen. Dat maakt het wonen daar nog leuker. Jenneke, wat zou het leuk zijn om in te toekomst nog eens samen visite te lopen zoals in Berekum 2000; dank voor je interesse in alle aspecten van dit project. Henne, ik vind leuk dat de vriendschap tussen onze families zijn oorsprong heeft in het Radboud. Richard, dank voor je interesse in mijn opleiding en voor de gesprekken over de raakvlakken van ons werk. Edith en Willem, dank voor jullie interesse in mijn promotietraject. Martijn, dank voor je gastvrijheid, en wellicht kom ik voor toekomstig onderzoek nog eens langs in Londen.

Mama en papa, dank voor het vertrouwen, de stimulans, interesse, relativering en steun op allerlei vlakken waardoor ik nu kan promoveren. Jullie waren en zijn een geweldig voorbeeld voor mij. Rian en Mechie, dank voor jullie interesse in mijn werk in het algemeen en in de voortgang van dit project.

Marie, jij kwam op het moment dat deze studie begon, en je hebt in het beloop van die 5 jaar ook veel geleerd over promoveren: "*Dan kunnen die andere dokters ervan leren, hè mama? Want jij weet dat allemaal al, en jij hebt het van Baziel geleerd, en Baziel heeft het weer van zijn baas geleerd, en die heeft het weer van zijn baas geleerd. En zo gaat het alsmaar door.*" Paul en Willem, jullie kwamen tegen het einde van dit onderzoek, en hebben leren lopen tijdens de discussie. Bij jullie moet ik dit alles even vergeten, en dat is prima. Theo, mede dankzij jou is deze klus geklaard: bedankt dat je er op ieder moment voor me bent. Ik hoop nog lang te genieten van je altijd goede humeur!

Donders Graduate School for Cognitive Neuroscience Series

1. van Aalderen-Smeets, S.I. (2007). *Neural dynamics of visual selection.* Maastricht University, Maastricht, the Netherlands.
2. Schoffelen, J.M. (2007). *Neuronal communication through coherence in the human motor system.* Radboud University Nijmegen, Nijmegen, the Netherlands.
3. de Lange, F.P. (2008). *Neural mechanisms of motor imagery.* Radboud University Nijmegen, Nijmegen, the Netherlands.
4. Grol, M.J. (2008). *Parieto-frontal circuitry in visuomotor control.* University Utrecht, Utrecht, the Netherlands.
5. Bauer, M. (2008). *Functional roles of rhythmic neuronal activity in the human visual and somatosensory system.* Radboud University Nijmegen, Nijmegen, the Netherlands.
6. Mazaheri, A. (2008). *The Influence of Ongoing Oscillatory Brain Activity on Evoked Responses and Behaviour.* Radboud University Nijmegen, Nijmegen, the Netherlands.
7. Hooijmans, C.R. (2008). *Impact of nutritional lipids and vascular factors in Alzheimer's Disease.* Radboud University Nijmegen, Nijmegen, the Netherlands.
8. Gaszner, B. (2008). *Plastic responses to stress by the rodent urocortinergic Edinger-Westphal nucleus.* Radboud University Nijmegen, Nijmegen, the Netherlands.
9. Willems, R.M. (2009). *Neural reflections of meaning in gesture, language and action.* Radboud University Nijmegen, Nijmegen, the Netherlands.
10. Van Pelt, S. (2009). *Dynamic neural representations of human visuomotor space.* Radboud University Nijmegen, Nijmegen, the Netherlands.
11. Lommertzen, J. (2009). *Visuomotor coupling at different levels of complexity.* Radboud University Nijmegen, Nijmegen, the Netherlands.
12. Poljac, E. (2009). *Dynamics of cognitive control in task switching: Looking beyond the switch cost.* Radboud University Nijmegen, Nijmegen, the Netherlands.
13. Poser, B.A. (2009) *Techniques for BOLD and blood volume weighted fMRI.* Radboud University Nijmegen, Nijmegen, the Netherlands.
14. Baggio, G. (2009). *Semantics and the electrophysiology of meaning. Tense, aspect, event structure.* Radboud University Nijmegen, Nijmegen, the Netherlands.
15. van Wingen, G.A. (2009). *Biological determinants of amygdala functioning.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
16. Bakker, M. (2009). *Supraspinal control of walking: lessons from motor imagery.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
17. Aarts, E. (2009). *Resisting temptation: the role of the anterior cingulate cortex in adjusting cognitive control.* Radboud University Nijmegen, Nijmegen, the Netherlands.
18. Prinz, S. (2009). *Waterbath stunning of chickens – Effects of electrical parameters on the electroencephalogram and physical reflexes of broilers.* Radboud University Nijmegen, Nijmegen, the Netherlands.
19. Knippenberg, J.M.J. (2009). *The N150 of the Auditory Evoked Potential from the rat amygdala: In search for its functional significance.* Radboud University Nijmegen, Nijmegen, the Netherlands.
20. Dumont, G.J.H. (2009). *Cognitive and physiological effects of 3,4-methylenedioxymethamphetamine (MDMA or 'ecstasy') in combination with alcohol or cannabis in humans* Radboud University Nijmegen, Nijmegen, the Netherlands.
21. Pijnacker, J. (2010). *Defeasible inference in autism: a behavioral and electrophysiogical approach.* Radboud Universiteit Nijmegen, the Netherlands.
22. de Vrijer, M. (2010). *Multisensory integration in spatial orientation.* Radboud University Nijmegen, Nijmegen, the Netherlands.
23. Vergeer, M. (2010). *Perceptual visibility and appearance: Effects of color and form.* Radboud University Nijmegen, Nijmegen, the Netherlands.
24. Levy, J. (2010). *In Cerebro Unveiling Unconscious Mechanisms during Reading.* Radboud University Nijmegen, Nijmegen, the Netherlands.
25. Treder, M. S. (2010). *Symmetry in (inter)action.* Radboud University Nijmegen, Nijmegen, the Netherlands.

26. Horlings C.G.C. (2010). *A Weak balance; balance and falls in patients with neuromuscular disorders.* Radboud University Nijmegen, Nijmegen, the Netherlands
27. Snaphaan, L.J.A.E. (2010). *Epidemiology of post-stroke behavioural consequences.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
28. Dado – Van Beek, H.E.A. (2010). *The regulation of cerebral perfusion in patients with Alzheimer's disease.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
29. Derks, N.M. (2010). *The role of the non-preganglionic Edinger-Westphal nucleus in sex-dependent stress adaptation in rodents.* Radboud University Nijmegen, Nijmegen, the Netherlands.
30. Wyczesany, M. (2010). *Covariation of mood and brain activity. Integration of subjective self-report data with quantitative EEG measures.* Radboud University Nijmegen, Nijmegen, the Netherlands.
31. Beurze S.M. (2010). *Cortical mechanisms for reach planning.* Radboud University Nijmegen, Nijmegen, the Netherlands.
32. van Dijk, J.P. (2010). *On the Number of Motor Units.* Radboud University Nijmegen, the Netherlands .
33. Lapatki, B.G. (2010). *The Facial Musculature – Characterization at a Motor Unit Level.* Radboud University Nijmegen, Nijmegen, the Netherlands.
34. Kok, P. (2010). *Word Order and Verb Inflection in Agrammatic Sentence Production.* Radboud University Nijmegen, Nijmegen, the Netherlands.
35. van Elk, M. (2010). *Action semantics: Functional and neural dynamics.* Radboud University Nijmegen, Nijmegen, the Netherlands.
36. Majdandzic, J. (2010). *Cerebral mechanisms of processing action goals in self and others.* Radboud University Nijmegen, Nijmegen, the Netherlands.
37. Snijders, T.M. (2010). *More than words – neural and genetic dynamics of syntactic unification.* Radboud University Nijmegen, Nijmegen, the Netherlands.
38. Grootens, K.P. (2010). *Cognitive dysfunction and effects of antipsychotics in schizophrenia and borderline personality disorder.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
39. Nieuwenhuis, I.L.C. (2010). *Memory consolidation: A process of integration – Converging evidence from MEG, fMRI and behavior.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
40. Menenti, L.M.E. (2010). *The right language: differential hemispheric contributions to language production and comprehension in context.* Radboud University Nijmegen, Nijmegen, the Netherlands.
41. van Dijk, H.P. (2010). *The state of the brain, how alpha oscillations shape behaviour and event related responses.* Radboud University Nijmegen, Nijmegen, the Netherlands.
42. Meulenbroek, O.V. (2010). *Neural correlates of episodic memory in healthy aging and Alzheimer's disease.* Radboud University Nijmegen, Nijmegen, the Netherlands.
43. Oude Nijhuis, L.B. (2010). *Modulation of human balance reactions.* Radboud University Nijmegen, Nijmegen, the Netherlands.
44. Qin, S. (2010) *Adaptive memory: imaging medial temporal and prefrontal memory systems.* Radboud University Nijmegen, the Netherlands.
45. Timmer, N.M. (2011). *The interaction of heparan sulfate proteoglycans with the amyloid β protein.* Radboud University Nijmegen, Nijmegen, the Netherlands.
46. Crajé, C. (2011). *(A)typical motor planning and motor imagery.* Radboud University Nijmegen, Nijmegen, the Netherlands.
47. van Grootel, T.J. (2011). *On the role of eye and head position in spatial localisation behaviour.* Radboud University Nijmegen, Nijmegen, the Netherlands.
48. Lamers, M.J.M. (2011). *Levels of selective attention in action planning.* Radboud University Nijmegen, Nijmegen, the Netherlands.
49. Van der Werf, J. (2011). *Cortical oscillatory activity in human visuomotor integration.* Radboud University Nijmegen, Nijmegen, the Netherlands.
50. Scheeringa, R. (2011). *On the relation between oscillatory EEG activity and the BOLD signal.* Radboud University Nijmegen, Nijmegen, the Netherlands.
51. Bögels, S. (2011). *The role of prosody in language comprehension: when prosodic breaks and pitch accents come into play.* Radboud University Nijmegen, Nijmegen, the Netherlands.
52. Ossewaarde, L. (2011). *The mood cycle: hormonal influences on the female brain.* Radboud University Nijmegen, Nijmegen, the Netherlands.

53. Kuribara, M. (2011). *Environment-induced activation and growth of pituitary melanotrope cells of Xenopus laevis.* Radboud University Nijmegen, Nijmegen, the Netherlands.
54. Helmich, R.C.G. (2011). *Cerebral reorganization in Parkinson's disease.* Radboud University Nijmegen, Nijmegen, the Netherlands.
55. Boelen, D. (2011). *Order out of chaos? Assessment and treatment of executive disorders in brain-injured patients.* Radboud University Nijmegen, Nijmegen, the Netherlands.
56. Koopmans, P.J. (2011). *fMRI of cortical layers.* Radboud University Nijmegen, Nijmegen, the Netherlands.
57. van der Linden, M.H. (2011). *Experience-based cortical plasticity in object category representation.* Radboud University Nijmegen, Nijmegen, the Netherlands.
58. Kleine, B.U. (2010). *Motor unit discharges - Physiological and diagnostic studies in ALS.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
59. Paulus, M. (2011). *Development of action perception: Neurocognitive mechanisms underlying children's processing of others' actions.* Radboud University Nijmegen, Nijmegen, the Netherlands.
60. Tieleman, A.A. (2011). *Myotonic dystrophy type 2. A newly diagnosed disease in the Netherlands.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.
61. van Leeuwen, T.M. (2011). *'How one can see what is not there': Neural mechanisms of grapheme-colour synaesthesia.* Radboud University Nijmegen, Nijmegen, the Netherlands.
62. van Tilborg, I.A.D.A. (2011). *Procedural learning in cognitively impaired patients and its application in clinical practice.* Radboud University Nijmegen, Nijmegen, the Netherlands.
63. Bruinsma, I.B. (2011). *Amyloidogenic proteins in Alzheimer's disease and Parkinson's disease: interaction with chaperones and inflammation.* Radboud University Nijmegen, Nijmegen, the Netherlands.
64. Voermans, N. (2011) *Neuromuscular features of Ehlers-Danlos syndrome and Marfan syndrome; expanding the phenotype of inherited connective tissue disorders and investigating the role of the extracellular matrix in muscle.* Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

Ehlers-Danlos syndrome (EDS) and Marfan syndrome are two of the most frequent inherited connective tissue disorders, characterized by joint hypermobility, tissue fragility and easy bruising, skin hyperextensibility, and / or arterial aneurysmata with ruptures. Neuromuscular features have been reported in incidental cases, and are generally ascribed to reduced physical activity.

Inspired by patients referred to us and by recent developments in neuromuscular research, we presented an overview of the clinical and molecular continuum of the inherited connective tissue disorders and certain myopathies. Next, we studied the occurrence and nature of neuromuscular features in EDS and Marfan syndrome, and showed that both disorders are associated with mild neuromuscular features, with signs of myopathy and polyneuropathy in EDS; and signs of myopathy, polyneuropathy, and lumbosacral radiculopathy in Marfan syndrome. Furthermore, we showed that severe fatigue and chronic pain are highly prevalent among EDS patients. The five possible determinants involved in fatigue in EDS are sleep disturbances, concentration problems, social functioning, self efficacy concerning fatigue, and pain severity. Pain is related to hypermobility, dislocations, and previous surgery and associated with moderate to severe impairment in daily functioning. Finally, we investigated the pathophysiological mechanisms of muscle weakness in EDS in order to explore the role of the extracellular matrix in muscle function. The results showed that muscle weakness in EDS is not caused by reduced physical activity but results from (1) alterations of the series elastic component of the myotendinous pathways (due to increased compliance of connective tissue of muscle and tendon); (2) a reduction of myofascial force transmission (due to increased compliance of connective tissue between muscles and fascia), due to which muscles act more independently; and (3) a failure to maximally voluntarily activate the muscles.

This study will probably increase awareness of the various neuromuscular features of these and other inherited connective tissue disorders among clinicians and researchers, and thus improve the clinical recognition of these symptoms. The results of the studies on fatigue and pain could form a starting point for the development of an effective cognitive behavioral intervention for fatigue in EDS. Treatment of pain should be a prominent aspect of the symptomatic management of EDS. The results have also raised new research questions, e.g. what is the occurrence of neuromuscular symptoms in other inherited connective tissue disorders; which pathophysiological mechanisms cause peripheral nerve dysfunction in EDS and Marfan syndrome; how do ECM defects in various types of EDS result in intracellular - both myopathic and axonal – changes; and why is central activation capacity reduced in EDS.